

Skeletal Deformities in Cultured Striped Catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) in the Mekong Delta, Vietnam

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Abstract

Skeletal deformities are a common issue that poses a challenge that hinders the production of cultured finfish worldwide. The present study aims to describe for the first time skeletal deformities in cultured striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) from the Mekong Delta, Vietnam. A total of 68 striped catfish were collected from three nurseries in highly concentrated areas of Long An and Dong Thap provinces. These provinces are known for their numerous hatcheries and nursing farms of *P. hypophthalmus* in the country. The skeletal deformities recorded included saddleback syndrome, missing pectoral fins, lordosis and kyphosis, unilateral deformities of the opercular complex bones, branchiostegal ray and lower jaw deformities. Abnormalities in bone morphotypes between normal and deformed fish were analysed using X-ray scanning and double staining (alizarin red and alcian blue) methods. The morphological abnormalities, in general, can reduce the fillet yield of fish, thereby affecting their market value. Since *P. hypophthalmus* is the most important freshwater fish exported from the Mekong Delta, a regular monitoring program of skeletal deformities can contribute to identifying improved rearing protocols and enhance the product quality available in the markets.

Keywords: Tra catfish, Mekong Delta, skeletal abnormalities, saddleback syndrome, X-ray

Introduction

Striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878), also known as Tra catfish or sutchi catfish, is a popular freshwater aquaculture species in many Asian countries. Its excellent growth rate and the ability to tolerate high stocking density and unfavourable environmental conditions have made this species a suitable candidate for farming. The fish is cultured in polyculture or mono-culture systems, in ponds, cages or pens at various scales ranging from small-scale commercial to large extensive-scale commercial operations. In 2018, farmed striped catfish global production was 2,359,500 tonnes, accounting for 4.3 % of total finfish production (FAO, 2020). Of these, 1,360,000 tonnes were produced by Vietnam, the world's largest producer of striped catfish (FAO, 2020).

One of the key drivers for the explosive growth of the

striped catfish in Vietnam is the success of artificial propagation (Phuong and Oanh, 2010) and the dissemination of the techniques throughout the Mekong Delta (De Silva and Phuong, 2011). In 2007–2008 the striped catfish hatcheries in the Mekong Delta had a capacity of approximately 818.3 million hatchlings (52 million fries), exceeding the farmer's demand (Le and Le, 2010). However, several factors have been identified as impacting the yield of nursing striped catfish. These include difficulties in brood stocks management (Nguyen, 2009), the impact of climate change (De Silva and Phuong, 2011), technological developments (Cacot, 1999; De Silva and Phuong, 2011) and the occurrence of disease issues (Hoa et al., 2021; Pokhrel and Oanh, 2021). Common pathogens associated with diseases in striped catfish culture include parasites, bacteria and fungi (Ly et al., 2009; Hoa et al., 2021). More recently, the emergence of skeletal malformations, particularly the saddleback syndrome appearing in fingerling striped

catfish, has been considered a new challenge to the development of this industry.

Skeletal abnormalities are a frequent problem in finfish aquaculture. These abnormalities can affect various parts of the fish's skeleton, the jaw bone, operculum, cranium, vertebral and fins (Divanach et al., 1996). In fin deformity, the malformation or the absence of one or all hard spines of the dorsal fin, accompanied by abnormality of the shape, position and number of the supporting pterygiophores (the internal bones that support the muscle and dorsal spines) (Tave et al., 1983; Koumoundouros et al., 2001) is called saddleback syndrome (SBS). In severe cases of SBS, the pectoral, pelvic or anal fins may also be affected (Tave et al., 1983). Skeletal deformities, including SBS, generally reduce the ability of fish to swim, catch prey, and digest food, hence likely increasing the suffering of fish from infectious diseases (Andrades et al., 1996; Imsland et al., 2006; Le Vay et al., 2007). In severe skeletal deformities, fish may die (Tave et al., 1983; Olsvik et al., 2021).

In contrast to the extensive research on skeletal abnormalities in various finfish, studies concerning skeletal deformities in catfish (Order Siluriformes) are limited. Skeletal abnormality has only been documented in a limited number of families within the order Siluriformes, including Bagridae, Callichthyidae, Clariidae, Heptapteridae, Ictaluridae, Loricariidae and Pimelodidae, accounting for only seven out of forty families in this order (Kusunoki and Ángel, 2017). Commonly reported skeletal abnormality of the spine in Siluriformes includes lordosis, characterised by excessive inward curvature, kyphosis, excessive outward curvature, and scoliosis with abnormal lateral curvature (Kusunoki and Ángel, 2017). In addition, skeletal deformity reported also includes deformity of the upper jaw and jawline and ankylosis, which is abnormal stiffening and immobility of joints due to the fusion of bones. To the best of authors' knowledge, there are no previous reports regarding skeletal abnormalities in the family *Pangasiidae*, particularly in the farmed striped catfish *P. hypophthalmus*. The present study aims to address this gap by reporting skeletal deformities in striped catfish fingerlings.

Materials and Methods

Ethical approval

Striped catfish samples and the methods used in this study were approved by the Animal Ethics Committees of Nong Lam University, under the approval of the project "Study on skeletal deformities in cultured striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) in the Mekong Delta, Vietnam", No. NLU-230101, dated 01 January 2022.

Specimen collection

From January to June 2022, normal and deformed

striped catfish aged between 20 and 90 days of culture were collected from three nursery farms in Long An and Dong Thap provinces in the Mekong Delta, of Vietnam. The total length, from the snout to the tip of the caudal fin and the standard length, from the snout to the posterior end of the last vertebra, were measured using a measuring board with an accuracy of 1.0 mm. The total weight for each fish was recorded to the nearest 0.01g using a digital analytical balance. The length, weight and origin of different fish groups are detailed in Supplementary Table. The specimens were fixed in 4 % formalin buffered to pH 7 with 0.1M phosphate buffer. After the preservation process, the specimens were transported to the Faculty of Fisheries laboratory, Nong Lam University, for further assessment.

X-ray scanning

The body and fins of the specimens were carefully examined for malformations and any other morphological anomaly. To further assess the internal skeletal structure, radiographs were taken using a portable digital X-ray apparatus (HI-Ray 100, Eickenmeyer Medizintechnik für Tierärzte e.K., Germany) and recorded on 30 Å~ 40 cm film (AGFA D7 DW, China).

Double staining

To confirm and assess the magnitude of the skeletal deformity in each fish, the alcian blue-alizarin red double staining method described by Darias et al. (2010) and Hanken and Wassersug (1981) was used with some modifications. In brief, fixed specimens were initially rinsed with distilled water before transferring into an alcian blue solution. The solution comprised 100 mg.L⁻¹ alcian blue 8GX (Sigma-Aldrich, USA), 800 mL.L⁻¹ 95 % ethanol, and 200 mL.L⁻¹ acetic acid. The specimens remained in the solution until the staining saturated the cartilage. The specimens were then incubated for 3 min in an acid-neutralising solution of 100 % ethanol in 1 % KOH. For rehydration, the specimens were subjected to two rounds of rehydration, with each step lasting 15 min, using a series of decreasing concentrations of ethanol (95, 70, 40, 15 %).

In the subsequent step, the specimens were cleaned with distilled water, followed by bleaching in a solution comprising 1 volume of 3 % H₂O₂ and 9 volumes of 1 % KOH. The ossified fish were then immersed in a rinsing solution composed of 7 volumes of distilled water, 3 volumes of sodium borate and 0.5–2.5 g trypsin (Sigma-Aldrich, USA) for 20 h. Subsequently, the specimens were incubated in alizarin red (Sigma-Aldrich, USA) solution of 5 g.L⁻¹ alizarin red in 1 % KOH for bone staining. The specimens were washed with distilled water and subsequently with a solution of 1 % KOH to eliminate any background staining. Finally, the specimens were dehydrated in glycerol and 1 % KOH before being

preserved in 100 % glycerol. The deformed specimens are deposited in the fish collection of the Department of Fish Pathology, Faculty of Fisheries, Nong Lam University, Vietnam.

Results

The study documented 68 striped catfish specimens with skeletal deformities collected from nursery farms using X-ray radiographs and double staining methods. Seven types of skeletal abnormalities were observed, as summarised in Table 1. Each abnormality was described in relation to the specific type of skeleton it affected.

Opercular deformity

The osteological investigation of the 68 abnormal specimens collected from three striped catfish nurseries in the Mekong Delta showed opercular deformity in 6 specimens (8.8 %). The opercular abnormalities in fingerling of striped catfish manifested at different levels, ranging from mild opercular deformation with moderate inward folding at the upper margin of the opercle to severely deformed operculum with extensive folding toward the interior of the gill cavity and resulting in a reduced operculum exposing a large portion of the gill filaments (Fig. 1d). Associated with the opercular malformation was the abnormality of surrounding cranial elements namely the branchiostegal rays (Fig. 1d) in addition to the lack of dorsal fin (Fig. 1c). In case of branchiostegal ray abnormality, there were cleft on the branchiostegal membrane and folding outward of the gill cavity (Fig. 1d). The severe opercular abnormalities showed partial to total absence of operculum leading to the exposed gill arches (Fig. 1d).

Lower jaw deformity

Lower jaw reduction was found in two striped catfish fingerlings (2.9 %), leading to the shortened lower jaw and its skew off-centre appearance together with the head. The fish's mouth cannot be closed (Fig. 2).

Fins deformity

Out of the 68 specimens collected, a significant

number of 50 samples (73.6 %) showed dorsal fin deformities closely associated with saddleback syndrome (SBS). Fish with SBS showed either a complete or partial absence of dorsal fin development, including the fin spine and fin rays (Figs. 3a, 3b). In several cases of dorsal fin abnormalities, the interneural and pterygiophores were absent (Figs. 3b, 3c). The complete absence of the pectoral fin on the right side was found in one fish with SBS (Fig. 3c).

Spinal column deformity

Three types of spinal abnormalities were observed in striped catfish: scoliosis, kyphosis, and lordosis. Scoliosis was the most predominant, accounting for 10.3 % of the specimens. It was characterised by lateral curvature of the spinal column (Figs. 4a, 4b). In some cases, multiple points along the spinal cord showed spinal curvature (Fig. 4b). Lordosis manifested as V-shaped dorsal-ventral curvature of the vertebral column in the sagittal orientation. While in kyphosis, it was seen as A-shaped dorsal-ventral curvature of the vertebral column (Figs. 4c, 4d). Both lordosis and kyphosis accounted for 4.4 % of the total specimens. And both lordosis and kyphosis resulted in compressed vertebrae and displayed anomalies in the neural and haemal arches of the affected regions (Fig. 4e). One specimen showed a combination of scoliosis and kyphosis (Fig. 4e). Fish with severe spinal column abnormalities usually showed abnormal anal fins (Fig. 4e).

Discussion

Deformities in fish primarily manifest as alterations in appearance and skeletal deformity, occurring in both wild (Matsuoka, 2003; Fjellidal et al., 2021) and farmed populations (Matsuoka, 2003; Hoyle et al., 2007; Fjellidal et al., 2021). Despite the increasing number of studies, the development of morpho-anatomical abnormalities remains a significant challenge that hinders the finfish aquaculture industry. The economic impact of skeletal abnormalities on European aquaculture is substantial, estimated at at least 50 million € annually. The frequency of deformities in Europe may vary with abnormalities' a mean ranging between 7-20 % and occasionally reaching up to 100 % (Boglione et al., 2013).

Table 1. Number (n) and per cent of different skeletal deformity types recorded in striped catfish, *Pangasianodon hypophthalmus* collected from three nurseries in Long An and Dong Thap provinces in the Mekong Delta, Vietnam.

No.	Skeletal deformity type	n	%
1	Operculum folded inwards and/or shorter operculum	5	7.3
2	Twisted branchiostegal rays associated with the deformed operculum	1	1.5
3	Lower jaw reduction	2	2.9
4	Lack of partial or total dorsal fins (saddleback syndrome)	49	72.1
5	Absence of pectoral fin (unilateral)	1	1.5
6	The lateral curvature spinal column (scoliosis)	7	10.3
7	V-shaped and A-shaped curvature of the vertebral column (lordosis and kyphosis)	3	4.4
	Total	68	100

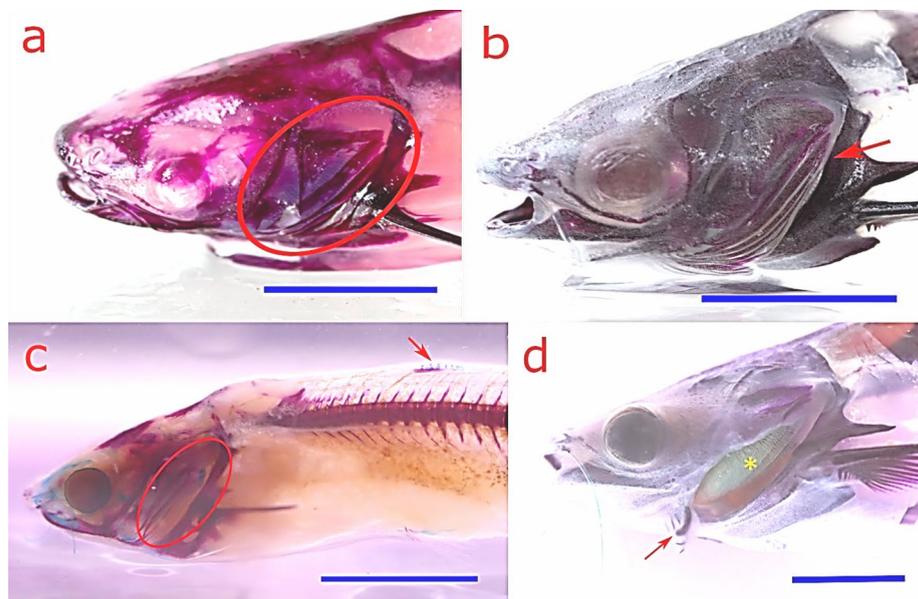


Fig. 1. Different levels of opercular malformation in striped catfish, *Pangasianodon hypophthalmus* fingerlings: (a) normal fish without any sign of opercular deformities; (b) mild opercular malformation with moderate inward folding at the upper margin of the operculum (arrow); (c) co-occurrence of opercular deformity and lack of dorsal fin; (d) severely deformed operculum with extensive folded toward the interior of the gill cavity showing the branchiostegal rays twisted (arrow) and gill arches (asterisk). Scale bars: 1 cm.

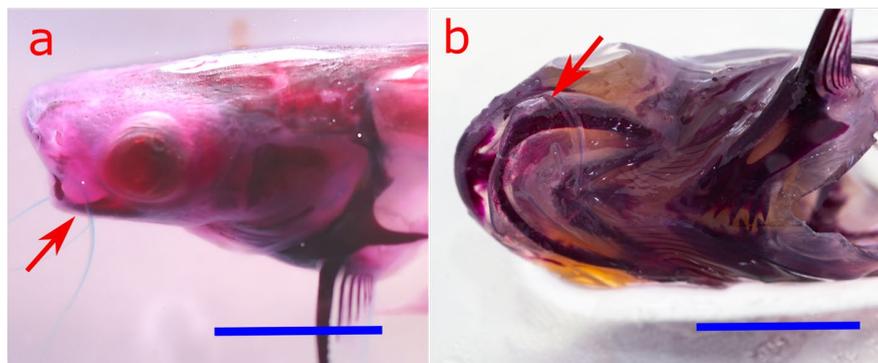


Fig. 2. Lower jaw reduction (arrow) in striped catfish, *Pangasianodon hypophthalmus*: (a) lateral view, (b) ventral view. Scale bars: 1 cm.

Recently, there have been reports of increasing frequency (up to 90 %) of presumptive SBS deformities observed and reported by many farmers from nurseries and over a much broader area in the Mekong Delta (Nguyen Thao Suong, pers. comm.). Although SBS alone does not appear to affect the survival of striped catfish, however, fish with SBS and other skeletal deformities such as missing pectoral fin or opercular deformities could experience difficulties in swimming performance and show increased susceptibility to diseases (Nguyen Thao Suong, pers. comm.). Currently, no data is available on the economic impact of SBS on striped catfish. However, SBS affects the product price of striped catfish resulting in a 10 % to 30 % reduction depending on market demand. Farmers often discard fish with SBS, hence downgrading hatcheries' production. Due to the severe implication of SBS, further research and analysis are required to understand the issue and its actual economic impacts on striped catfish.

The cause of skeletal deformities in fish is complex, involving many factors. In their review study, Eissa et al. (2021), stated that skeletal deformities in wild or farmed fish could result from environmental pollutants, infectious agents, dietary deficiencies, genetics and rearing conditions, such as thermal shock and overcrowding. Regarding nutrition, Haga et al. (2011) reported that deficiency or excess of vitamin A can result in bone deformities. In addition, phosphorus, calcium, vitamins C and D and essential fatty acids have also been reported to affect skeletal development (Eissa et al., 2021). Other possible causes of skeletal deformities in fish could be tank colour (Cobcroft and Battaglione, 2009), sensitivity to the rearing process, and human errors during the rearing process (Boglione et al., 2013). In the present study, the fish were fed daily with a 100 % commercial diet consisting of 30 % to 40 % crude protein at 3 % to 8 % of their body weight fed two or more times daily, depending on their size which is considered as standard feeding regime for nursing striped catfish.

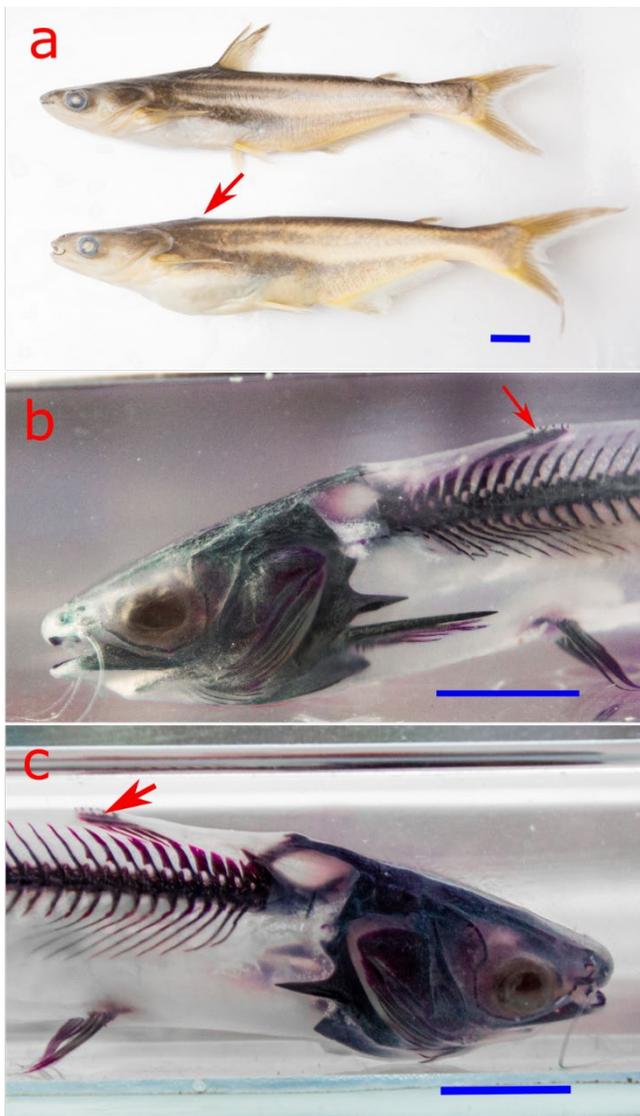


Fig. 3. Fins deformities in striped catfish, *Pangasianodon hypophthalmus*: (a) macroscopic photographs of the normal specimen (without an arrow) and deformed fish without the dorsal fin (arrow); (b) the total absence of dorsal fin formation (arrow); (c) the complete lack of the pectoral fin in conjunction with the saddleback syndrome (arrow). Scale bars: 1 cm.

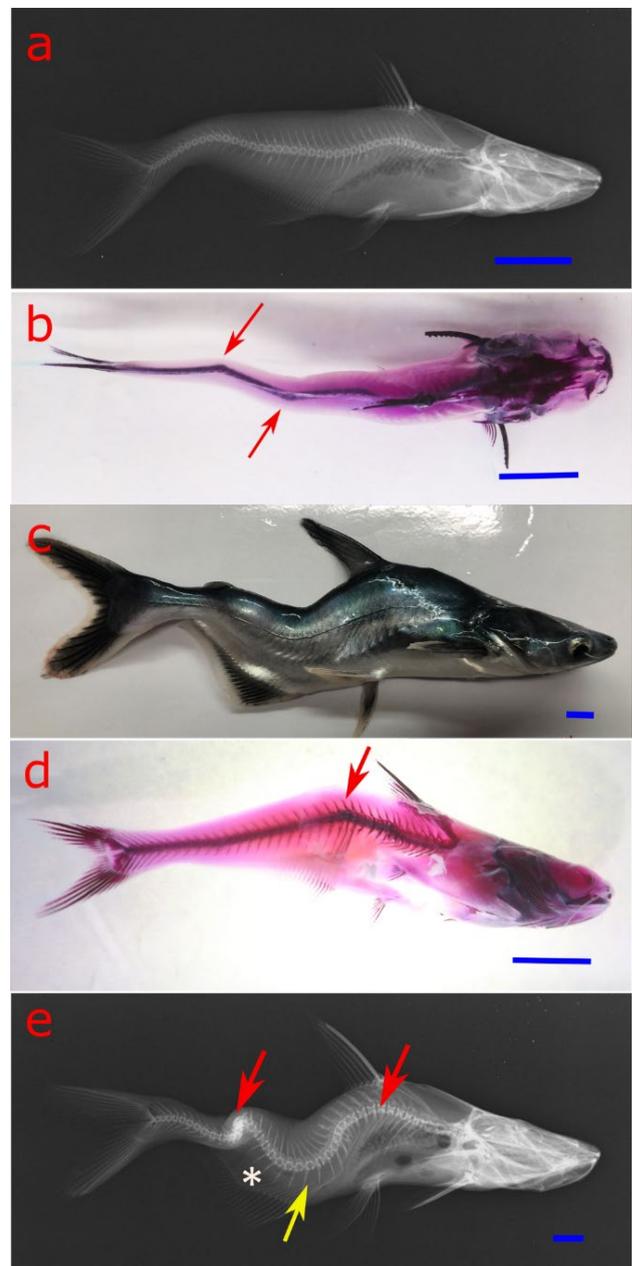


Fig. 4. Vertebral column anomalies in striped catfish, *Pangasianodon hypophthalmus*. The lateral curvature of spinal column - scoliosis showing two curvature positions (arrow) along the axis column (a & b). The V-shaped dorsal-ventral curvature of the vertebral column - lordosis and the A-shaped dorsal-ventral curvature of the vertebral column - kyphosis (arrow) (c & d). The co-occurrence of lordosis (yellow arrow), kyphosis (red arrow) and deformed anal fin (asterisk) showing compression vertebrae with neural and haemal arches anomalies at the alteration regions (e). Scale bars: 1 cm.

The exact cause of each type of deformity may differ in each case and may be challenging to determine (Jawad et al., 2019). As in the case of *Clarias gariepinus* (Burchell, 1822), reviewed by Kusunoki and Ángel (2017), the causes of lordosis in larvae were due to malathion exposure (Liem et al., 1997); however, the causes of lordosis and kyphosis in adults were not determined (Eissa et al., 2009). In contrast, a report by Alarape et al. (2015) indicated that the causes of lordosis and kyphosis in adult *C. gariepinus* could be multifactorial.

Skeletal deformities in farmed fish can occur at any stage of the life cycle, from the larvae stage (López-Albors et al., 1995; Lein et al., 1997; Kestemont et al., 2007) to the adult stage (Andrades et al., 1996; Korsøen et al., 2009). The present study identified seven types of skeletal deformities in striped catfish fingerlings which included the SBS, absence of pectoral fin, lordosis and kyphosis, unilateral deformities of the opercular complex bones, branchiostegal ray deformities and lower jaw

reductions. Details of size, origin and skeletal deformities associated with each striped catfish sample are provided in Supplementary Table. The striped catfish hatcheries in Vietnam produce a significant number of larvae, of which 94 % are sold to nursery farms. The nursery farm then grows the fry into fingerlings for sale to the grow-out sectors (Phuong et al., 2013). However, not much is known whether these seven types of deformities that occur early at the larval stage in striped catfish would be seen in adult fish. Cahu et al. (2003) found that spinal curvature remains unchanged or worsens with age. Therefore, a systematic surveillance and monitoring program in striped catfish from the hatching stages may help fill the knowledge gap concerning the deformity-specific size for improved rearing protocols and enhanced product quality at markets.

Fish with skeletal deformity experience many challenges, varying according to the seriousness of the anomaly. Skeletal anomalies in fish not only affect their physical structure but also have detrimental impacts on their biological performance. It could affect the swimming and feeding behaviour if it involves the fins. Serious jaw anomalies impair efficiency in feeding with consequences on the growth rate. (Boglione et al., 2013). According to Paperna et al. (1980) (cited by Boglione et al., 2013), gill cover anomalies have been linked to increased susceptibility to oxygen stress and myxobacterial infections as well as associated with negative growth rate during the larval stage (Boglione et al., 2013). In gilthead seabream, *Sparus aurata* Linnaeus, 1758, a decrease in the occurrence of gill cover during metamorphosis suggests selective mortality of deformed fish. Pre-haemal kyphosis in European seabass, *Dicentrarchus labrax* (Linnaeus, 1758), leads to lethargic behaviour and high mortality due to the compression of the neural tube by deformed vertebrae during vertebral axis osteogenesis. Lordosis has been found to significantly reduce endurance and critical swimming speed in European seabass juveniles, as demonstrated by Basaran et al. (2007). These findings highlight the significant consequences of skeletal deformities on fish health and performance. Therefore, it is necessary to understand the factors contributing to fish deformities to develop effective strategies for prevention and management to minimise the high economic losses to the aquaculture industry.

Conclusion

Striped catfish *Pangasianodon hypophthalmus* is considered a significant species farmed in the Mekong Delta, Vietnam and globally. However, skeletal deformities in this species are not well understood. In the current study, seven types of skeletal deformities in striped catfish fingerlings were observed, including saddleback syndrome, absence of pectoral fin, lordosis and kyphosis, unilateral deformities of the opercular complex bones,

branchiostegal ray deformities and lower jaw reduction. To better comprehend and minimise the losses to the aquaculture industry, future research should investigate the causes and effects of these deformities on striped catfish.

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Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Nguyen Thao Suong: Conceptualisation, writing - original draft. Truyen Nha Dinh Hue: Conceptualisation, writing - review and editing. Mai Dang Tien: Conceptualisation, writing - review and editing. Nguyen Huu Thinh: Conceptualisation, writing - review and editing. Nguyen Phuc Thuong: Conceptualisation, methodology development, writing - review and editing.

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Supplementary Table. Size, origin and skeletal deformity type of striped catfish, *Pangasianodon hypophthalmus* fingerlings collected from Mekong Delta.

Fish no.	Standard length(cm)	Total length(cm)	Origin	Type of skeletal deformity
1	15.3	18.7	Dong Thap	Operculum fold inwards
2	14.2	17.2	Dong Thap	
3	15.3	18.8	Dong Thap	
4	2.49	3.15	Long An	
5	2.34	2.76	Long An	
1	15.4	17.6	Dong Thap	Branchiostegal rays twisted associated to deformed operculum
1	15.26	18.53	Long An	Lower jaw reduction
2	10.73	13.15	Long An	Lack of partial or total of the dorsal fins (saddleback syndrome)
1	2.22	2.53	Long An	
2	1.71	1.94	Long An	
3	1.81	2.13	Long An	
4	2.07	2.32	Long An	
5	1.55	1.79	Long An	
6	1.81	2.08	Long An	
7	1.77	2.1	Long An	
8	2.48	2.91	Long An	
9	1.65	1.84	Long An	
10	1.72	2.03	Long An	
11	1.59	1.91	Long An	
12	1.62	1.87	Long An	
13	1.66	1.9	Long An	
14	1.74	1.95	Long An	
15	1.81	2.08	Long An	
16	1.62	1.84	Long An	
17	1.74	2.06	Long An	
18	1.68	1.94	Long An	
19	2.19	2.67	Long An	
20	1.76	2.11	Long An	
21	1.74	2.06	Long An	
22	1.22	1.43	Long An	
23	1.89	2.23	Long An	
24	1.67	1.95	Long An	
25	1.6	1.87	Long An	
26	1.69	2.08	Long An	
27	1.37	1.58	Long An	
28	1.62	1.85	Long An	
29	1.55	1.73	Long An	
30	1.71	1.92	Long An	
31	1.38	1.62	Long An	
32	1.45	1.72	Long An	

Supplementary Table. Continued.

Fish no.	Standard length (cm)	Total length (cm)	Origin	Type of skeletal deformity
33	1.51	1.79	Long An	
34	1.8	1.95	Long An	
35	1.67	1.95	Long An	
36	1.47	1.71	Long An	
37	10.7	12.1	Dong Thap	
38	9.5	11.4	Dong Thap	
39	9.3	10.9	Dong Thap	
40	11.8	13.7	Dong Thap	
41	9.4	11.4	Dong Thap	
42	9.8	11.6	Dong Thap	
43	11.2	12.4	Dong Thap	
44	12.5	15.4	Dong Thap	
45	9.8	11.9	Dong Thap	
46	9.4	11.7	Dong Thap	
47	10.8	12.9	Dong Thap	
48	11.8	14.1	Dong Thap	
49	13.2	15.9	Dong Thap	
1	10.1	12.3	Long An	Absent of pectoral fin (unilateral) and saddleback syndrome
1	6.47	7.15	Long An	The lateral curvature spinal column (scoliosis)
2	9.8	12.1	Dong Thap	
3	8.4	10.3	Dong Thap	
4	9.8	11.8	Dong Thap	
5	3.5	5.4	Long An	
6	3.8	5.6	Long An	
7	4.2	6.7	Long An	
1	15.26	18.53	Long An	V-shaped and Λ -shaped curvature of the vertebral column (lordosis and kyphosis)
2	18.7	22.1	Long An	
3	20.4	24.2	Dong Thap	