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Vertebral deformities in hatchery-reared and wild-caught juvenile Japanese flounder, *Paralichthys olivaceus**

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Abstract The present study compared vertebral deformities of hatchery-reared and wild-caught juvenile Japanese flounder, *Paralichthys olivaceus*. A total of 362 hatchery-reared flounder (total length 122.5–155.8 mm) were collected from three commercial hatcheries located in Yantai, East China, and 89 wild fish (total length 124.7–161.3 mm) were caught off Yangma Island near Yantai City (37°27′N, 121°36′E). All the fish were dissected, photographed, and images of the axial skeleton were examined for vertebral deformities. Compared with wild-caught flounder in which no deformed vertebrae were detected, 48 (13.3%) hatchery-reared fish had deformed vertebrae. The deformities were classified as compression, compression-ankylosis, and dislocation-ankylosis. The vertebral deformities were mainly localized between post-cranial vertebra 1 and 3, with vertebrae number 1 as the most commonly deformed. The causative factors leading to vertebral deformities in reared Japanese flounder may be related to unfavorable temperature conditions, inflammation, damage, or rupture to the intervertebral ligaments under rearing conditions. Furthermore, no significant difference in the total number of vertebral bodies was observed between wild-caught (38.8±0.4) and hatchery-reared flounder (38.1±0.9) (P>0.05). However, the number of vertebral bodies of hatchery-reared and wild-caught flounder ranged from 35 to 39 and from 38 to 39, respectively.

Keyword: *Paralichthys olivaceus*; hatchery-reared; wild-caught; deformities; vertebrae

1 INTRODUCTION

Skeletal deformities have been reported in many commercially important farmed fish. These skeletal deformities include abnormalities of the vertebral column/axial skeleton (Madsen and Dalsgaard, 1999; Fjelldal et al., 2007a, 2009a), the jaw and operculum (Haga et al., 2003; Fraser and De Nys, 2005), fin supports (Boglione et al., 2001), and the caudal bone complex (Deschamps et al., 2008; Haga et al., 2011). The causative factors leading to skeletal deformities in fish have been associated with nutritional imbalances (Fraser et al., 2004; Haga et al., 2011), unfavorable biotic/abiotic conditions (Eissa et al., 2009; Sánchez et al., 2011), infection (Ben Alaya et al., 2011), environmental pollution (Teh et al., 2002; Davidson et al., 2011), and genetic variation (Shikano et al., 2005; Gislason et al., 2010).

The occurrence of skeletal deformities has resulted in severe economic losses for the aquaculture industry due to the reduced market value of the affected products, as well as the additional labor cost of manual sorting out deformed fish (Hattori et al., 2003). In addition to the direct economic losses, skeletal deformities have also been related to high mortality (Puvanendran et al., 2009; Ben Alaya et al., 2011), limited feeding and swimming behavior (Le Vay et al., 2007; Davidson et al., 2011), reduced growth and feeding efficiency (Eissa et al., 2009; Fjelldal and Hansen, 2010), and susceptibility to infectious disease (Davidson et al., 2011).

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The Japanese flounder, *Paralichthys olivaceus*, has been used for marine aquaculture and stock enhancement in China and Japan for the past three decades (Katayama and Isshiki, 2007; Lv et al., 2011). Since the problem of skeletal deformities in hatchery-reared Japanese flounder was initially reported (Takeuchi et al., 1998), several studies have focused on the development of different jaw deformities, as these are one of the best described and conspicuous skeletal deformities in cultured flounder (Haga et al., 2002, 2003). Deformities of the vertebral column are another very common problem in hatchery-reared Japanese flounder juveniles under extensive and intensive rearing conditions. Vertebral deformities are observed in the larval phase of cultured fish species (Fraser and De Nys, 2005) and can be manifested as several types, including (1) compression, (2) compression-ankylosis/fusion, and (3) ankylosis/fusion-dislocation of the normal vertebral column (Witten et al., 2005; Berg et al., 2006; Fjelldal et al., 2006). Few studies have addressed deformities in the vertebral column of Japanese flounder and a lack of knowledge is available regarding the types and occurrence of vertebral deformities in hatchery-reared and wild-caught individuals of this species. Therefore, we investigated, characterized, and compared the vertebral deformities observed in hatchery-reared and wild-caught Japanese flounder juveniles.

2 MATERIAL AND METHOD

2.1 Fish sampling

This study was conducted at the Fish Behavioral Ecology Laboratory of the Ocean University of China from July to August 2012. Japanese flounder juveniles were collected from field and commercial hatcheries. All fish were divided into four groups and two categories (Table 1); (1) wild-caught fish (group I) collected from landings of set-net and beam-trawl fisheries near the shores of Yangma Island (37°27′N, 121°36′E, Shandong Province, China) in July 2012, (2) hatchery-reared fish (including groups II, III, and IV) collected from hatcheries at Yantai (Shandong Province, China) during July and August 2012.

2.2 Vertebral column observations

Total length (to the nearest 0.1 mm) and body weight (to the nearest 0.1 g) were measured (Table 1) before fish were immersed in 10% formalin. Individuals with external signs of vertebral deformities were recorded and photographed. Each flounder within the hatchery-reared and wild-caught groups was dissected on the ocular side in a glass dish and photographed under artificial light with a digital camera attached to a binocular dissecting microscope.

The images were used to analyze vertebral deformities (internal morphology) to determine (1) the total number of vertebral bodies (for fish with vertebral deformities, the total number before deformities occurred), (2) whether or not there were deformed vertebrae, (3) the number of individuals with deformed vertebrae, (4) the number of deformed positions for each individual, (5) the location of the deformed vertebrae on the vertebral column, and (6) the types of vertebral deformities observed.

Three types of vertebral deformities (Fjelldal et al., 2007a) were used to classify the observed deformities: (1) compression, (2) compression-ankylosis, and (3) ankylosis-dislocation. A compressed vertebra (type 1) had a normal central part and deformities in the cranial and caudal parts. Vertebrae that were compressed in the cranio-caudad direction with intervertebral spaces (without central fusion) were classified as the compressed. Compression-ankylosis (type 2) deformities were characterized by a fusion of two or more deformed vertebral bodies. Adjacent vertebrae that were compressed in the cranio-caudad direction and lacked intervertebral spaces (with central fusion) were classified as ankylosis-compression. Ankylosis-dislocation (type 3) deformities were characterized by a lateral dislocation between adjacent normal vertebrae that lacked intervertebral spaces (with central fusion).

2.3 Data analysis

The number of vertebrae is presented as mean±standard deviation for all Japanese flounder juveniles. Student's *t*-test was used to examine the differences between wild-caught and hatchery-reared fish. The data from the wild-caught group and the three hatchery-reared groups were compared by one-way analysis of variance. Multiple comparisons between means were performed using Duncan's test to detect significant differences ($P<0.05$).

3 RESULT

3.1 The number of vertebral bodies

No significant difference was observed in the total number of vertebral bodies between wild-caught (38.8±0.4, n=89) and hatchery-reared (38.1±0.9, n=362) flounder ($P>0.05$). In addition, no significant

Table 1 Source of juvenile Japanese flounder, *P. olivaceus*, and sampling information

	Wild-caught fish	Hatchery-reared fish		
Group	I	II	III	IV
Sampling location	Yangma Island	Zhifu	Haiyang	Muping
Sampling date	July 2012	July 2012	July 2012	August 2012
Range of total length (mm)	124.7–161.3	129.2–155.8	122.5–151.8	125.8–149.2
Range of body weight (g)	14.8–34.9	18.8–34.2	15.5–32.7	16.4–30.9
No. of dissected fish	89	87	217	58
Total	89		362	

Table 2 Vertebrae in juvenile Japanese flounder, *P. olivaceus*

	Wild-caught fish	Hatchery-reared fish		
Group	I	II	III	IV
Range of vertebral bodies	38–39	36–39	35–39	35–39
Total	38–39		35–39	
No. of vertebral bodies (mean±S.D.)	38.8±0.4	37.9±0.7	38.2±0.8	38.0±1.0
Total	38.8±0.4*		38.1±0.9*	
No. of fish with vertebral deformities (%)	0 (0)	9 (10.3)	32 (14.7)	7 (12.1)
Total (%)	0 (0)		48 (13.3)	

*: Numbers are average values.

Table 3 The number of individuals with deformed vertebrae (*n*=48) and the distribution of the three types of vertebral deformities (*n*=72) in the three groups of hatchery-reared Japanese flounder

Category	Group	Number of deformed vertebras				Total number of deformed positions [a]	Types of deformities [b]		
		1	2	3	4		C	CA	DA
	II	7	2	0	0	11	3	8	0
Hatchery-reared fish	III	22	5	3	2	49	13	32	4
	IV	4	1	2	0	12	1	10	1
Total (%)		33 (68.8)	8 (16.7)	5 (10.4)	2 (4.1)	72	17 (23.6)	50 (69.4)	5 (7.0)

[a] The total number of deformed positions means that these deformed positions are on deformed vertebrae of hatchery-reared flounder; [b] C: compression; CA: compression and ankylosis; DA: dislocation and ankylosis.

difference in vertebral body number was found among the hatchery-reared groups of flounder (37.9±0.7, 38.2±0.8, and 38.0±1.0 for the three hatchery-reared groups, respectively; $P>0.05$; Table 2). The number of vertebral bodies in wild-caught flounder ranged from 38 to 39, and that of hatchery-reared flounder ranged from 35 to 39.

3.2 Location and number of deformed vertebrae

Compared to the wild-caught juvenile flounder group (group I), in which no deformed vertebrae were observed, all three hatchery-reared groups had a high prevalence of individuals with deformed vertebrae; nine (10.3%), 32 (14.7%), and seven (12.1%), respectively, for groups II, III, and IV (Table 2). Of the 362 hatchery-reared flounder, 48 (13.3%) had deformed vertebrae.

Among the 48 flounder with deformed vertebrae, the number of deformed positions per fish ranged from 1 to 4. There were 33 (68.8%) individuals with one deformed position, eight (16.7%) with two deformed positions, five (10.4%) with three deformed positions, and two (4.1%) with four deformed positions (Table 3). Thus, a total of 72 deformed positions were observed among the 48 flounder with deformed vertebrae.

The vertebral column was divided into three regions to study the exact location of deformed vertebrae along the vertebral column (Fig.1). Vertebral bodies were encoded (1–39) from the post-cranial region to the postero-caudal region of the vertebral column. The results indicated that deformed vertebrae were not distributed randomly. Using the morpho-functional division suggested by Meunier and Ramzu (2006), vertebral deformities were found mainly in the post-cranial region, and only a few vertebral deformities were found in the middle and postero-caudal regions of the axial skeleton. In the post-cranial region, 39/72 (54.2%) of the deformed vertebrae were found between vertebral body numbers 1 and 3 (Fig.1).

3.3 Types of deformed vertebrae

All three types of vertebral deformities (compression, compression and ankylosis, and dislocation and ankylosis) were found only in hatchery-reared Japanese flounder (Fig.2b–d). The distribution of the three types of vertebral deformities

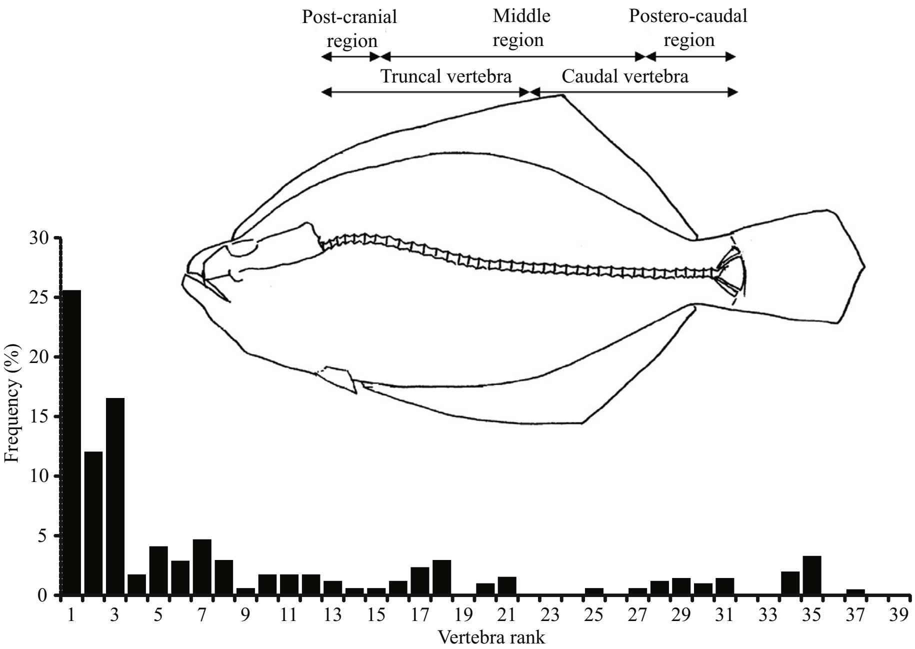

Fig.1 Schematic drawing of the Japanese flounder skeleton showing the vertebral regionalization defined by Meunier and Ramzu (2006) and the frequency (%) of deformed vertebrae along the axial skeleton (*n*=72)

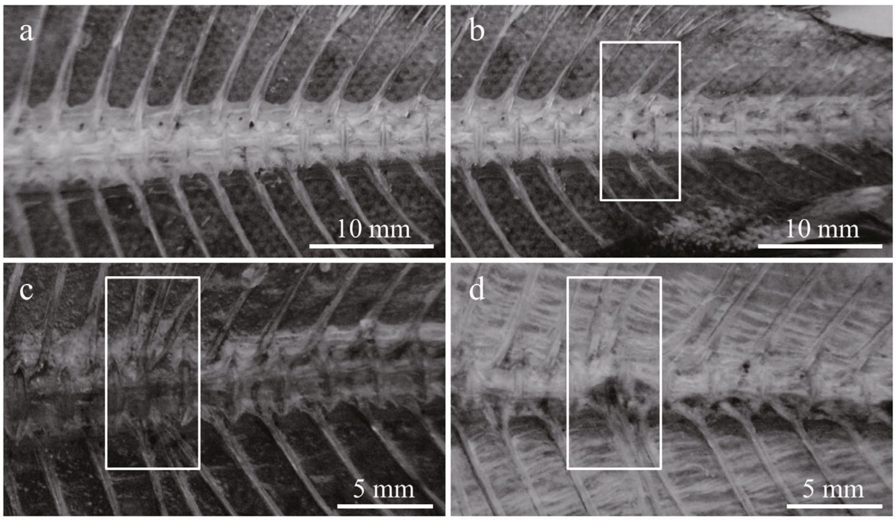

Fig.2 Vertebral photographs of dissected Japanese flounder

a. Normal vertebra; b. "compression" deformity in the vertebral column; c. "compression-ankylosis" deformity in the vertebral column; d. "ankylosis-dislocation" deformity in the vertebral column.

is shown in Table 3. Within the 48 deformed flounder with a total of 72 deformed vertebrae, 14 fish had vertebral compression with 17 (23.6%, *n*=72) deformed positions, 45 fish had vertebral compression and ankylosis with 50 (69.4%, *n*=72) deformed positions, and five fish had vertebral dislocation and ankylosis with five (7.0%, *n*=72) deformed positions (Table 3). Moreover, no fish with external signs of vertebral deformities was observed among hatchery-reared or wild-caught Japanese flounder during the experimental period.

4 DISCUSSION

4.1 Number of vertebral bodies

Our results clearly demonstrate no significant difference in the total number of vertebral bodies between wild-caught and hatchery-reared juvenile flounder. It has been suggested that vertebral meristic characters are established during the early developmental stage of fish and as early as embryogenesis (Tåning, 1952; Fowler, 1970; Lewis and Lall, 2006). This observation suggests that the number of vertebrae is established during the embryonic stage and before hatching (Fowler, 1970).

However, genetic and environmental factors are known to affect the number of meristic characters during pre-fertilization and early developmental periods (Fowler, 1970; Dentry and Lindsey, 1978). Parameters such as photoperiod and temperature, specifically temperatures below the optimal level for a given species, result in higher variability in the number of meristic characters during key developmental periods (Jordan, 1891). Lewis et al. (2004) described the total number of vertebral bodies for hatchery-reared juvenile Atlantic halibut, *Hippoglossus hippoglossus*, and their results demonstrated that morphological variation among hatchery-reared individuals increased because of differences in water temperature during early development. For example, the number of vertebral bodies within a specific region increased, but no significant difference was observed in the total number of vertebral bodies (Lewis et al., 2004). In addition, comparative studies between cultured and wild gilthead sea bream, *Sparus aurata* L., larvae revealed that cultured individuals have more variable meristic characters than those of wild fish (Boglione et al., 2001). Similarly, in the current study, the number of vertebral bodies in wild-caught flounder ranged from 38 to 39, whereas the number of vertebral bodies in hatchery-reared flounder ranged from 35 to 39. As mentioned above, the higher variability in meristic characters in cultured fish compared to wild fish may be due to differences and diversity in environmental factors under culture conditions.

4.2 Occurrence of vertebral deformities

No wild-caught Japanese flounder were found with vertebral deformities. However, approximately 4% of wild-caught gilthead sea bream display one or two vertebral body deformations (Boglione et al., 2001). Fjelldal et al. (2009a) reported that the occurrence of vertebral deformities in wild-caught Atlantic cod, *Gadus morhua* L., based on radiological examinations was approximately 6%, which was approximately 5–13 times lower than that of hatchery-reared individuals. Similar results have been reported for red sea bream, *Pagrus major*, (Matsuoka, 1987) and herring, *Clupea harengus*, (Balbontin et al., 1973). Furthermore, much higher frequencies of vertebral deformities than those we observed in wild-caught individuals have only been observed in polluted waters (Westernhagen et al., 1988; Whittle et al., 1992) or in freshwater, which is subjected to significant variations in environmental parameters such as temperature (Hubbs, 1959). Both Shelbourne (1964) and Vladimirov (1975) proposed the hypothesis of severe selection pressure against deformed fish in the wild, but this has not been demonstrated experimentally.

While there are reports of deformed larvae occurring in the natural environment, the level of deformities in wild fish is low compared to that in farmed larval and juvenile fish (Komada, 1980; Boglione et al., 2001). In this study, the prevalence of vertebral deformities in hatchery-reared Japanese flounder was 13.3%. A higher prevalence of deformities among cultured individuals has been reported in several species; 61%–80% in Atlantic halibut (Lewis and Lall, 2006), 40%–85% in Atlantic salmon, *Salmo salar* (Fjelldal et al., 2009a), and 34%–95% in European sea bass, *Dicentrarchus labrax* (Barahona-Fernandes, 1982; Boglione et al., 2001). The results indicate that Japanese flounder are not as susceptible as other cultured species to develop vertebral deformities. As there may be high variability in the number of deformed individuals between or within fish farms (Deschamps et al., 2008), it would not be suitable to collect fish from only one farm to compare with wild fish. For this reason, the hatchery-reared Japanese flounder used in this study were collected and analyzed as three hatchery-reared groups.

4.3 Types of vertebral deformities

Vertebral deformities were classified into three types in this study, and each of these types was observed within each group of hatchery-reared Japanese flounder. The types of vertebral deformities observed were similar to those reported in other studies (compression, compression-ankylosis, and dislocation-ankylosis) (Wargelius et al., 2005; Witten et al., 2005; Berg et al., 2006; Fjelldal et al., 2007a, 2009a). Fjelldal et al. (2009a) reported that some vertebral bodies of Atlantic cod with a normal morphology could develop as the compressed, compressed and ankylosed, or dislocated and ankylosed, as evidenced by radiographs taken at 1-year intervals. In addition, a previous radiological study of cultured Atlantic salmon showed that compressed vertebrae developed from normal vertebrae or less compressed vertebrae, and that ankylosed and compressed vertebrae developed from ankylosed and dislocated vertebrae (Fjelldal et al., 2007a).

We found that deformed Japanese flounder possessed one or more of the deformed types of vertebrae, and that the most frequently observed vertebral deformity was compression and ankylosis. Wargelius et al. (2005) suggested that there are two main types of vertebral deformities in cultured fish: one originating from changes in the intervertebral spaces and another originating from changes in the growth zones of the vertebrae. Changes in the intervertebral spaces, which may be related to inflammation (Kvellestad et al., 2000) and/or damage to or rupture of the intervertebral ligaments (Fjelldal et al., 2004), may be manifested on radiographs as ankylosis and dislocation. Changes in the growth zones of vertebrae may be related to linear growth zone failure in the compact bone of the vertebra (Nordvik et al., 2005) caused by soft bone (Fjelldal et al., 2007b), which is manifested as compression on radiographs. If these changes are also responsible for vertebral deformities in hatchery-reared Japanese flounder, further study is needed to investigate how the different types of vertebral deformities occur and develop in this species.

4.4 Location of vertebral deformities

Vertebral deformities may develop in different regions of the vertebral column. In this study, the deformities were mainly localized between post-cranial vertebra 1 and 3, with vertebra number 1 (25.6%) as the most deformed vertebra. In Atlantic salmon parr, Fjelldal et al. (2007b) found that deformities were located in the area between vertebra numbers 1 and 16, whereas Sullivan et al. (2007) found that the predominant location for vertebral deformities was between vertebra numbers 21 and 27. In harvest size Atlantic salmon, Berg et al. (2006) found that the predominant location for vertebral deformities was vertebra numbers 50 to 58, whereas Fjelldal et al. (2009b) found that vertebra numbers 31 to 49 were most often deformed, with vertebra number 43 as the most deformed vertebra. In larval barramundi, *Lates calcarifer*, Fraser et al. (2004) found that the predominant location for vertebral deformities was vertebra numbers 16 to 24, which are located in the caudal area of the vertebral column. Similarly, Gavaia et al. (2002) reported that 44% of deformities occurred in the caudal vertebrae of the Senegal sole, *Solea sengalensis*. Based on the locations of the deformities in these previous studies and the present study, it is suggested that deformed vertebrae are not randomly distributed. However, it is apparent that vertebral deformities may occur/develop in different regions of the vertebral column in different fish species and even during different life stages of the same species.

5 CONCLUSION

A higher prevalence of vertebral deformities was found in hatchery-reared juvenile Japanese flounder compared to wild-caught flounder. The deformities included compression, compression-ankylosis, and dislocation-ankylosis. These deformities were mainly localized between post-cranial vertebra 1 and 3, with vertebra number 1 as the most deformed vertebra. The total number of vertebral bodies was not significantly different between wild-caught and hatchery-reared juvenile Japanese flounder. The vertebral deformities observed in this study may have implications for Japanese flounder feeding, growth, and biology. The development of vertebral deformities and the causative factors inducing these deformities have been well described for some fish species such as Atlantic cod, Atlantic salmon, and Atlantic halibut. However, very few studies on the vertebral deformities of Japanese flounder have been reported. This preliminary study provides information on skeletal morphology of Japanese flounder and identifies the need for additional fundamental research. Further investigation is needed to understand the factors responsible for the development of vertebral deformities in this species.

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