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Morphological and osteological malformations in hatchery bred redline torpedo fish, *Sahyadria denisonii* (Day 1865) (Cyprinidae)

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Resumen

Malformaciones morfológicas y oseas en ejemplares de criadero de barbo de línea roja, Sahyadria denisonii *(Day 1865) (Cyprinidae)*

Se publican, por primera vez, malformaciones morfológicas y osteológicos de *Sahyadria denisonii* producidas en criadero. Se fotografiaron anomalías morfológicas con cámara digital y de rayos X digital. De 950 larvas producidas, sólo 12 individuos (1,26 %) presentaron algún tipo de anomalía, incluyendo lordosis, escoliosis, cifosis, semi-opérculo, deformidad de la aleta, deformidad de la boca, deformación de la cabeza y deformidades múltiples. La causa exacta de anomalías no se pudo determinar, pero se discuten las posibles etiologías. Sin embargo, el registro actual es significativo debido a la aparición de deformidades en peces ornamentales de agua dulce de criadero, para los que se considera la reproducción inducida como la clave de su conservación.

Palabras clave: Deformidad, Reproducción inducida, Radiografía, Ghats Occidentales.

Abstract

This is the first published report on morphological and osteological malformations in hatchery produced *Sahyadria denisonii*. Morphological abnormalities were photographed with a digital camera and digital X-ray. In present study, out of 950 larvae produced only 12 individuals (1.26%) were found to have any type of abnormality, including lordosis, scoliosis, kyphosis, semi-operculum, fin deformity, mouth deformity, head deformity and multiple type deformities. The exact cause of abnormalities could not be definitively determined, but the possible aetiologies are discussed. The present record is, nevertheless, significant owing to the occurrence of deformity in hatchery produced young ones of a freshwater ornamental fish for which induced breeding is considered to be the key to conservation.

Key words: Deformity, Induced breeding, Radiography, Western Ghats.

Introduction

Deformities in fish are relatively well described with a higher frequency of occurrence reported in hatchery produced individuals than wild (Sennar 1980, Hosoya & Kawamura 1998, Ma *et al.* 2014). Deformities in fishes are known to be caused as a result of environmental contaminants, scarcity of nutrients, oxygen deficiency, sudden changes in temperature, water current, mutation, inbreeding, parasitic infestation, mechanic trauma, and attack from predators (Fagbuaro 2009, Amitabh & Firoz 2010, Tave *et al.* 2011, Malekpouri *et al.* 2015). Numerous reports are available regarding abnormalities associated with both freshwater and marine fish species (Poynton 1987, Avyle *et al.* 1989, Panday & Awasthi 1994, Madsean & Dalsgard 1999, Raj *et al.* 2004, Olatunji-Akioye *et al.* 2010, Dutta *et al.* 2011, Jaward & Mamry 2012, Ma *et al.* 2014, Sajan *et al.* 2014, Malekpouri *et al.* 2015). *Sahyadria denisonii* (Day 1865), is popularly known as Redline torpedo fish or Miss Kerala and is endemic to the fourteen rivers flowing through the Western Ghats of India (Mercy *et al.* 2013a, Sajan 2015). This species is much sought after in the international ornamental fish trade (Raghavan *et al.* 2007, Mercy *et al.* 2010, Mercy *et al.* 2015) and it contributes the major share of India's ornamental fish exports during 2005-2012 (Raghavan *et al.* 2013). As a result of targeted and indiscriminate exploitation of this species for aquarium trade from wild listed those into endangered group. To the best of our knowledge, occurrence of any kind of deformities in hatchery produced *S. denisonii* has not yet been reported. This paper documents for the first time different types of deformities recorded in hatchery produced *S. denisonii*.

Material and methods

Induced breeding and larval rearing

Ripe *S. denisonii* were anaesthetised to minimise handling stress (Sajan *et al.* 2012) and induced bred at fish hatchery of College of Fisheries, Kerala, India (Mercy *et al.* 2015). The newly hatched larvae (Average size=3.50±0.20mm) consume its yolk up to 4 days of post hatch, and then paramecium culture was given as the first exogenous feed. After a week of larval husbandry, they were weaned with live micro worms (*Panagrellus redivivus* (Goodey, 1945)) and followed by Artemia Flakes (OSI feeds, U.S.A) and commercial formulated diet (Higahsi Aqua feeds Pvt. Ltd) with crude protein (38 %), crude fat (4 %), crude fibre (3 %), ash (16 %) and moisture (11 %). The formulated feed was given twice a day *ad libitum* throughout the study period, the unconsumed feeds and excrements were siphoned out from the tanks and 1/3 portion of the water in each tanks was exchanged before next feeding (Mercy & Sajan 2014). Water quality parameters such as temperature, dissolved oxygen, pH, alkalinity, hardness and ammonia were daily monitored by following standard procedures (APHA 1992) and maintained within optimum ranges.

Examination of deformities

950 hatchery bred larvae were examined and abnormal specimens were anaesthetized by using MS-222 (Mercy *et al.* 2013b). Morphological abnormalities were photographed with a digital camera (Nikon Coolpix L22) and it was further examined with digital X-ray system (Fujifilm FCR Capsula XL II Reader). Morphological, anatomical terminologies relating to the fish abnormality were used by following Al-Harbi (2001). We selected all the deformed fish that could be observed in that particular breeding batch. For the comparison of abnormality, a normal specimen was also radiographed. The deformed specimens were preserved in 4% formaldehyde solution and deposited in the Museum of Department of Fishery Biology, College of Fisheries, KUFOS, Panangad, Kerala, India (FRM-SAH-DEN/2013-2).

Results

In present study, out of 950 larvae produced only 12 individuals (1.26 %) were found to have any type of abnormality. These abnormal fishes were grown up to an average length of 5.60 ± 0.84 cm in the hatchery compared to normal specimen (6.40 ± 0.53 cm), before they were sampled for the study (4-6 month of age). Length and weight of deformed specimens except those affected semi-operculum were measured. The water quality parameters were recorded as temperature (27.0 ± 0.50 ºC), pH (7.0±0.3), dissolved oxygen (5.29 ± 0.24 ppm), alkalinity (35.6 ± 6.0 ppm), hardness (55.46 ± 10.28 ppm) and ammonia (<0.02 ppm). Different types of morphological as well as osteo-

logical malformations recorded in *S. denisonii* were listed in Table. 1. In present study, malformations were externally apparent compared to normal specimens (Figs. 1, 2) and observed deformities include semi-operculum, vertebral deformity, head deformity, mouth deformity, fin deformity and also multiple deformities.

Type of deformity	Occurrence (%)
Lordosis	12.9
Scoliosis	9.68
Kyphosis	3.23
Semi-operculum	6.45
Fin deformity	22.6
Mouth deformity	9.68
Head deformity	9.68
Multiple deformity	25.8

Tabla 1. Porcentaje de ocurrencia de diferentes tipos de deformidad en *S. denisonii*.
Table 1. Percentage of occurrence of different types of deformity in *S. denisonii*.

Figura 1. Ejemplares de criadero de *S. denisonii* con anomalías.
Figure. 1. Hatchery produced *S. denisonii* with abnormalities.

Vertebral malformations were recorded by the presence of excessive inward curvature and abnormal lateral curvature respectively (Fig. 3). Scoliosis was affected the vertebral column at the post dorsal fin region of *S. denisonii* (Fig. 4) had total length of 5.8 cm (Body weight of 2.16 g.) compared to normal fish had total length of 6.2 cm (Body weight of 2.15 g). In the present study, vertebral deformities were frequently affected in the posterior half of the spinal column (Figs. 3c, 3d). Meanwhile one abnormal fish was noticed with lordosis and kyphosis (Fig. 3c). Abnormal fishes had functional double chambered swim-bladder (Figs. 3c, 3d), similar to normal specimen (Fig. 2b).

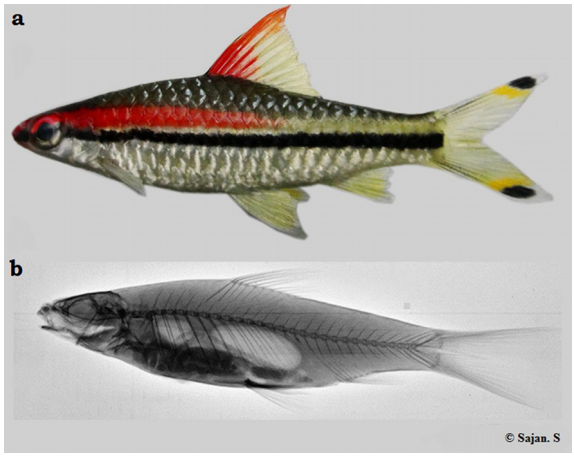

Figura 2. a: Espécimen normal de *S. denisonii*. anomalías. **b:** Radiografía.
Figure 2. a: Normal specimen of *S. denisonii*. **b:** X-ray image.

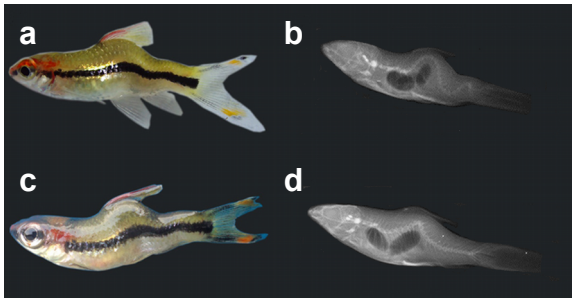

Figura 3. a, b: Ejemplares de *S. denisonii* con lordosis. **c-d:** Radiografías mostrando lordosis y cifosis.
Figure 3: a, b: *S. denisonii* showing lordosis. **c-d:** X-ray image showing lordosis and kyphosis.

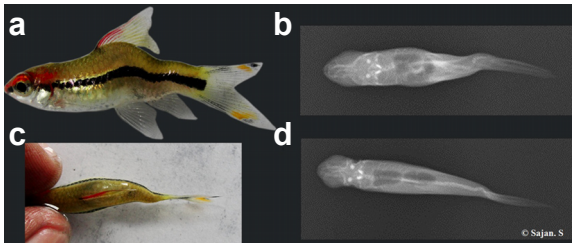

Figura 4. a, b: Ejemplares de *S. denisonii* mostrando escoliosis. **c, d:** Radiografías mostrando escoliosis.
Figure 4. a, b: *S. denisonii* showing scoliosis. **c, d:** X-ray image showing scoliosis.

Normal specimen of *S. denisonii* has mouth at sub-terminal position (Figs. 2a, 2b), while malformed specimens had depressed lower and upper jaw (Fig. 5b). Head of deformed specimen appeared as laterally compressed and shortened (Fig. 5a) compared to normal specimen (Figs. 2a, 2b). Type of head deformities like beak-head, inward bending of the lower jaw and shortening of the neurocranium were also noticed in *S. den-*

Figura 5. a: *S. denisonii* mostrando deformidad en cabeza. **b:** Deformidad en boca. **c, d:** Radiografías mostrando deformidades en boca y cabeza. **e, f:** Vista dorsal de espécimen normal.

Figure 5. a: *S. denisonii* showing head deformity. **b:** Showing mouth deformity. **c, d:** X-ray image showing mouth and head deformity. **e,f:** Dorsal view of normal specimen.

isonii (Figs. 5c, 5d). A case of unilateral semi-operculum was observed in *S. denisonii* by the shortening of the posterior part of operculum (Figs. 6a, 6b). Fin abnormalities were mainly affect the dorsal-caudal fins (Fig. 7a) and abnormal caudal fin observed as truncated towards the posterior side (Figs. 7a, 7b). More than one deformities (Multiple deformity) were recorded in eight abnormal specimens like semi-operculum, spinal deformity, head deformity, mouth deformity or fin deformity in one sample itself (Figs. 1, 6b).

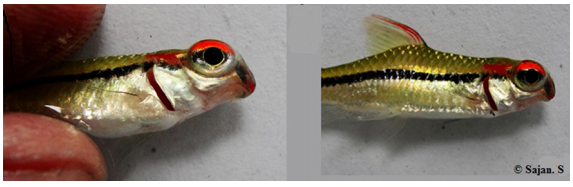

Figura 6. a,b: *S. denisonii* mostrando deformidad de semi-opérculo.

Figure 6. a,b: *S. denisonii* showing operculum deformity

Figura 7. a: *S. denisonii* mostrando deformidad en aletas caudal y dorsal. **b:** Radiografía mostrando deformidad en aletas caudal y dorsal.

Figure 7. a: *S.denisonii* showing caudal and dorsal fin deformity **b:** X-ray image showing caudal and dorsal fin deformity.

Discussion

Information's on body malformation is an important biological aspect in hatchery production of fish larvae. Larvae of several freshwater fish produced through induced breeding exhibit morphological abnormalities that adversely affect their survival rate (Daoulas *et al.* 1991, Gavaia *et al.* 2002, Sahoo *et al.* 2004, Sahoo *et al.* 2007, Boglione *et al.* 2013). Available evidence suggests that abnormalities are induced during the embryonic and post-embryonic periods of life (Al-Harbi 2001). In present study, we describes different abnormalities have been recorded in hatchery produced *S. denisonii*.

Occurrence of vertebral deformities are more common in early stages as compared to adults, which may be attributed to that deformed fish at early stages were more prone to mortality (Amitabh & Firoz 2010). Vertebral deformities like lordosis and scoliosis was recorded in *S. denisonii* by the presence of excessive inward curvature and abnormal lateral curvature respectively. According to Boglione *et al.* (1993), scoliosis is the lateral bending of the vertebral axis, it is the most easily distinguishable abnormality in live fishes. *S. denisonii* with scoliosis had two curvatures in the vertebral column at the post dorsal region, one below the dorsal fin region and second at the caudal peduncle region. In present study, vertebral deformities were more affected generally at the posterior half of the vertebral column. Similar types of vertebral deformities were reported in *Fundulus heteroclitus* (L., 1766) (Gabriel 1944) and *Esox lucius* L., 1758 (Orska 1962). Recently, wild caught specimen of *S. denisonii* from River Valapattanam, Kerala was also recorded with vertebral deformity (Sajan *et al.* 2014).

Spinal deformities were found to be associated with the absence of a functional swim-bladder (Iseda *et al.* 1979, Kitajima *et al.* 1981, Daoulas *et al.* 1991, Chatain 1994, Andrades *et al.* 1996). In the present study, deformed fish had normal

double chambered swim-bladder similar to Cyprinus carpio L., 1758 (Al-Harbi 2001) and *Labeo rohita* (Hamilton, 1822) (Dutta *et al.* 2013). Fluctuation in water temperature is considered to be one of the causes of the spinal deformities, because by the sudden change in water temperature may lead to abnormal muscle growth and spinal deformity (Al-Hassan 1982, Wang & Tsai 2000, Davidson *et al.* 2011). Low dissolved oxygen content in the water during spawning and developmental stages may also responsible for vertebral deformity (Al-Hassan 1982). But in the present study, dissolved oxygen content may never a limiting factor, because continuous aeration was provided in the hatching and rearing tanks to maintain dissolved oxygen level. The water quality parameters were maintained at optimum level, so in present study it is unable to correlate deformities with water quality parameters.

In present study, average length and weight of ten abnormal fish have been found to be substantially lower than that of normal fishes, probably due to their inability to feed normally and compete with the normal ones for food (Al-Harbi 2001). Dabrowski *et al.* (1988) and Frischkenecht *et al.* (1994) reported vitamin-C deficiency in the diet also leads to vertebral deformities. Finally, a genetic basis has also been proposed for spinal deformations (Fagbuaro 2009, Arbuatti *et al.* 2013), vertebral deformities are known to be hereditary and non- hereditary (Yamamoto *et al.* 1963). Vertebral abnormalities are also known to occur in *Cirrhinus mrigala* (Hamilton, 1822) and *Hypothalmichthys motitrix* (Valenciennes, 1844) (Raj *et al.* 2004) and *Poecilia wingei* Poeser, Kempkes & Isbrücker, 2005 (Arbuatti *et al.* 2013) due to inbreeding depression. In present study deformed fishes were not analysed genetically, hence it could not be ascertained whether the anomalies were hereditary or non-hereditable. Mouth abnormalities of various types have been reported in *Dicentrarchus labrax* (L., 1792) (Barahona-Fernades 1982) and *C. carpio* (Al-Harbi 2001). The mouth position of normal specimen of *S. denisonii* is sub-terminal, but the deformed specimens had depression on their lower and upper jaw of the mouth. Jaw deformity is known to be caused by many factors such as mechanical injury, nutritional deficiency, environmental condition, parasitism or genetic aberration (Quigley 1995). Beak like appearance of head, inward bending of the lower jaw and shortening of the neurocranium were also noticed in malformed *S. denisonii*. According to Al-Harabi (2001) distended or compressed head of deformed fishes may be due to the ossification or compression of bones.

Operculum related malformations are attributed to inside or outside folding, shortening or abnormal positioning of the opercular and subopercular bones, bilaterally or unilaterally (Boglione *et al.* 1993, Galeotti *et al.* 2000). In present study, unilateral semi-operculum malformation was observed in *S. denisonii*. Commonly abnormality is seen only on one side of the operculum (unilateral), while some species had bilateral semi-operculum (Al-Harbi 2001). Operculum deformity has also been reported in hatchery raised *Oreochromis niloticus* (L., 1758) (Mair 1992), *Oreochromis mossambicus* (Peters, 1852) (Handwerker & Tave 1994). Vitamin-C deficiency related operculum deformity has been reported in *Oncorhynchus mykiss* (Walbaum, 1792) (Frischknecht *et al.* 1994) and *C. carpio* (Dabrowski *et al.* 1988). Fishes with such deformity can swim normally, but their growth was less than that of compared to the normal fish (Al-Harbi 2001).

Dorsal and caudal fin abnormalities were recorded in deformed specimen of *S. denisonii*. Similar types of fin disorders have also been observed in *Pampus argenteus* (Euphrasén, 1788) (Mamry *et al.* 2010), *C. mrigala* (Dutta *et al.* 2011) and *Moolgarda pedaraki* (Valenciennes, 1836) (Jaward & Mamry 2012). According to Boglione *et al.* (1993), fin anomalies are frequently observed in hatchery reared fish, but their frequency and severity vary according to the species, rearing condition or types of rearing tank. Some of the deformed specimen of S. denisonii had multiple deformities, such as semi-operculum, spinal deformity, head deformity, mouth deformity and fin deformity in one fish, similar reports by Tave *et al.* (1982) in *Oreochromis aureus* (Steindachner, 1864) (=*Sarotherodon aureus* (Steindachner, 1864)), Dabrowski *et al.* (1988) in *C. carpio*, Wiegand *et al.* (1989) in *Carassius auratus* L., 1758 and Frischknecht *et al.* (1994) in *O. mykiss*.

Fish deformities have been also resulted from nutritional deficiencies, unfavourable abiotic factors, rearing conditions and genetic factors (Yamamoto *et al.* 1963, Tave *et al.* 1982, Dab-

rowski *et al.* 1988, Boglione *et al.* 1993, Frischknecht *et al.* 1994, Wang & Tsai 2000, Fagbuaro 2009, Amitabh & Firoz 2010, Arbuatti *et al.* 2013). However, earlier studies reported that the possible aetiologies and mechanisms responsible are not well understood (Koumoundouros *et al.* 1997, Gavaia *et al.* 2002). This study has attempted to provide insights into the morphological and osteological deformities in hatchery produced young ones of *S. denisonii*. We could not observe any deformed fish that died before we commenced our investigation. However, we are not sure whether we had overlooked any while in the larval stage. This is not a problem in the hatchery since we got good survival rate. This is reported as a case report as it is the first of its kind. *S. denisonii* is a difficult to breed fish and the captive breeding technology of the species has been developed first by the authors (Mercy *et al.* 2015).

The present study does not mean to discuss the causes of the abnormalities, but only to single out the fact that such deformities are recorded in the hatchery-reared *S. denisonii*. Even though the exact cause of deformity was not determined in the present study, unfavourable abiotic conditions, inappropriate nutrition, genetic defects, disruption of early developmental process or a combination of these factors could all have been involved in the malformations in *S. denisonii*. Therefore, more research is needed to exactly identify the factors causing such deformities. However, the present record is nevertheless significant owing to the occurrence of deformity in hatchery produced young ones of a highly sought after freshwater ornamental fish for which captive breeding is considered to be the key to conservation and sustainable use.

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