Skeletal deformities in larval, juvenile and adult stages of cultured gilthead sea bream (Sparus aurata L.)

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Abstract

Gilthead sea bream (Sparus aurata L.) is currently farmed in a number of European countries. In culture exploitations, spinal malformations are frequently seen in adult specimens. We studied skeletal deformities in larval, juvenile and adult stages of fish from a Spanish experimental culturing centre. About 27% of sea bream larvae at hatching showed different types of axial deformations that were related to notochord alterations during embryogenesis. About 22% died soon after hatching, but 5% survived and reached juvenile and adult stages. These fish were mostly lordotic. Juvenile lordotic fish displayed uninflated swimbladders but all lordotic adults possessed an inflated functional swimbladder. Lordosis is characterised by V-shaped dorsoventral curvature of the body axis including the vertebral column and the spinal cord. Spinal curvature occurred more frequently between vertebrae 10 and 16. The congenital or postnatal origin of lordosis is discussed.

Keywords: Skeletal malformation; Spinal deviation; Lordosis; Hatchery; X-ray; Swimbladder; Notochord; Larva; Juvenile; Sparus aurata

1. Introduction

Gilthead sea bream is a species of great importance in Mediterranean aquaculture. The high level of spinal malformations appearing in hatchery fish is an important
problem for the development of this industry. This is often associated with growth depression, leading to high mortality rates (Barbaro et al., 1984; Shimizu, 1987).

The aetiology of these syndromes is not well understood. Nutritional, environmental and genetic causes have been cited. Alterations associated with deficiencies in ascorbic acid, tryptophan, phospholipid or vitamin D in the diet of parentals, have been shown to result in different types of skeletal deformities in descendants (Weis and Weis, 1989; Akiyama et al., 1989; Hinton et al., 1992). Consanguinity favoured accumulation of genetic abnormalities (Kincaid, 1976; Paperna, 1978; Piron, 1978). Environmental factors such as density of eggs, mechanical or thermal shocks, presence of pollutants in the water, radiation, salinity, oxygen depletion and light intensity have also been reported to cause aberrations in development (Haya, 1989; Wiegand et al., 1989; Caris and Rice, 1990).

Some authors have reported that spinal malformations in teleosts may be a consequence of an inability to inflate the swimbladder (SB) (Kitajima et al., 1981; Chatain, 1987; Daoulas et al., 1991; Chatain, 1994). Lordosis in Japanese sea bream (Chrysophrys major) (Kitajima et al., 1977; Kitajima, 1978; Iseda et al., 1979; Kitajima et al., 1981), gilthead sea bream (Sparus aurata) (Paperna, 1978; Chatain, 1994), and sea bass (Dicentrarchus labrax) (Chatain, 1994), has been correlated with the absence of a functional swimbladder. In some cases, lordosis induced by such behaviour has been reported to be partly or totally corrected following late inflation of the swimbladder (Kitajima et al., 1977).

How and when spinal deformities appear are, at the moment, unanswered questions. In this study we carried out a morphological description of lordotic adults and juveniles of S. aurata. We have also studied larval populations from hatching to 60 days as different axial deformities appear at this stage.

2. Materials and methods

2.1. Larvae

Larvae were maintained in three tanks of 5000 l volume in a flow-through system (temperature 18 ± 2°C, salinity 35‰, dissolved O₂ 95% saturation, photoperiod 12:12 LD) and fed on enriched rotifers (Brachionus plicatilis) from days 4–5 to days 25–35 after hatch, and brine shrimp (Artemia) starting from days 12–15 after hatch. From each tank, five samples of 300 ml each were taken randomly at different zones and levels from the rearing tank at all experimental times. After anaesthesia with MS-222 (Sandoz) (0.02 g l⁻¹), larvae were counted under a stereomicroscope. An estimation of the number of larvae in each tank was made by extrapolation of the data. The final number is the mean of estimates for the three tanks. One minute before sampling, aeration was stopped. No differences were found between the different regions of the tank or between the three tanks. Mortality was estimated by the daily decrease in the number of larvae during the experimental period (60 days). The standard length (from mouth to tail tip) was measured under a stereomicroscope for each sample. The percentage of deformed larvae was estimated by counting 300 larvae for each experimental period.
For histological observation, normal and deformed larvae were fixed by immersion in Bouin’s fixative, dehydrated in alcohol, embedded in butyl-methyl-methacrylate resin, sectioned (1–2 μm) and stained with toluidine blue. A Nikon Microphot-FXA microscope was used.

2.2. Adult and juveniles

Normal and lordotic (n = 50), adult and juvenile gilthead sea bream (Sparus aurata) of both sexes were provided by an experimental fish culturing centre (PEMARES, El Toruño, Cádiz, Spain). The specimens were anaesthetised, weighed and radiographed using a medical X-ray system. The X-ray pictures were used for examination of the skeleton and swimbladder. The degree of lordosis was evaluated by measuring the angle between the line from the first vertebra in the head region to the curvature point and the line from the curvature point to the last vertebra in the tail region (Chatain, 1994; 

Fig. 1. X-ray radiographs of a normal (a) and a lordotic (b) fish showing a V-shaped vertebral column (grade of deformation, α, is 45°). The vertex occurs between vertebrae 9 and 11. Dorsal spines in the curvature region appear fused (arrow). SB, swimbladder. Magnification × 0.8.
Andrades et al., 1994). Adults and juvenile animals were fed ad libitum with Callista chione and Loligo sp.

3. Results

3.1. Adults and juveniles

Lordotic adult sea bream displayed large heads in relation to their stout, compressed bodies, that appeared deviated in the dorsal and ventral zones. The caudal fin ascended and the lateral line showed an irregular trajectory. Such fish were shorter than normal. X-ray examination (Fig. 1(b)) showed V-shaped dorsoventral curvature of the vertebral column. In acute cases, vertebrae in the curvature region had fused neural spines.

Fig. 2. X-ray radiographs of juvenile normal (a) and lordotic (b, c) fish. Lordotic fish lack an inflated swimbladder (SB). The degree of deformation of the vertebral column in lordotic animals varies. Note in (c) several points of curvature in the vertebral column (arrows). Magnification × 1.5.
(xypholordosis) and showed hypercalcification. Affected haemal spines were bent by abdominal muscle pressure. In other fish a couple of ribs, often near the head, appeared fused (brachyspondilosis). All adults examined, either normal (Fig. 1(a)) or lordotic, displayed a functional swimbladder.

In juvenile animals, skeletal signs of lordosis were similar to those in adults but frequently more than one point of curvature was identified. Fig. 2(b) shows a major lordotic curvature (about 45°), between vertebrae 10 and 13, and another curvature in the distal caudal vertebrae. Some specimens showed such slight undulating curvatures of the vertebral column that externally they were recognizable only by the irregular trajectory of their lateral lines (Fig. 2(c)). Indeed, observation of the lateral line is a useful method to identify low lordotic curvatures in juvenile fish. In contrast to normal juveniles (Fig. 2(a)), lordotic juveniles showed uninflated swimbladders (Fig. 2(b) and (c)) and hence negative buoyancy. Fish had to swim constantly to avoid falling to the bottom of the tank. These fish showed slanted, nervous and jerky movements and high voracity although their growth rate was lower than that of normal fish (see below). They also were more susceptible to trauma (i.e. transport, anaesthesia and time out of the water to be radiographed). After a period of 3 months in the laboratory, all lordotic juveniles developed an inflated swimbladder and then displayed normal swimming behaviour, although lordosis persisted.

Juvenile and adult S. aurata have 24 spinal vertebrae (four cervical, 12 abdominal and eight caudal). We estimated the frequency each vertebra was the vertex of a curvature. Vertebrae 10–16 (abdominal and caudal) were most frequently affected by

Table 1
Age, cumulative and daily mortality, standard length (SL, mean ± SD) in normal and deformed larvae and estimated number of individuals during larval period, in hatchery-reared gilthead sea bream

<table>
<thead>
<tr>
<th>Days post-hatching</th>
<th>No. of larvae a (×10³)</th>
<th>Cum. mortality of all larvae (%)</th>
<th>Daily mortality of all larvae (%)</th>
<th>Deformed larvae (%)</th>
<th>SL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>797</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>2.75 ± 0.11</td>
</tr>
<tr>
<td>1</td>
<td>773</td>
<td>3</td>
<td>3</td>
<td>23</td>
<td>3.20 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>750</td>
<td>5.9</td>
<td>3</td>
<td>21</td>
<td>3.30 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>720</td>
<td>9.6</td>
<td>4</td>
<td>21</td>
<td>3.30 ± 0.16</td>
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<tr>
<td>4</td>
<td>684</td>
<td>14.1</td>
<td>5</td>
<td>18</td>
<td>3.40 ± 0.19</td>
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<tr>
<td>5</td>
<td>636</td>
<td>20.2</td>
<td>7</td>
<td>19</td>
<td>3.50 ± 0.22</td>
</tr>
<tr>
<td>6</td>
<td>591</td>
<td>25.7</td>
<td>7</td>
<td>18</td>
<td>3.60 ± 0.27</td>
</tr>
<tr>
<td>7</td>
<td>544</td>
<td>31.7</td>
<td>8</td>
<td>16</td>
<td>3.70 ± 0.33</td>
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<tr>
<td>8</td>
<td>489</td>
<td>38.5</td>
<td>10</td>
<td>16</td>
<td>3.80 ± 0.41</td>
</tr>
<tr>
<td>9</td>
<td>440</td>
<td>44.6</td>
<td>9.9</td>
<td>16</td>
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<tr>
<td>10</td>
<td>418</td>
<td>47.4</td>
<td>5</td>
<td>13</td>
<td>4.41 ± 0.58</td>
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<tr>
<td>20</td>
<td>293</td>
<td>63.2</td>
<td>30</td>
<td>11</td>
<td>5.92 ± 0.84</td>
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<tr>
<td>30</td>
<td>234</td>
<td>70.5</td>
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<td>7.53 ± 0.84</td>
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<tr>
<td>40</td>
<td>211</td>
<td>73.5</td>
<td>9.9</td>
<td>6</td>
<td>10.37 ± 1.33</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
<td>74.4</td>
<td>4.9</td>
<td>6</td>
<td>13.26 ± 1.53</td>
</tr>
<tr>
<td>60</td>
<td>190</td>
<td>76.1</td>
<td>4.9</td>
<td>5</td>
<td>15.11 ± 1.28</td>
</tr>
</tbody>
</table>

a Estimated.
lordosis, with a maximum value at vertebra 12. The lowest frequency corresponded to the vertebrae behind the head or in front of the tail.

3.2. Larvae

Initially, each 5000 l tank contained about 800,000 larvae. The highest mortality occurred during the first month (Table 1). Larvae reabsorbed the yolk sac and start

Fig. 3. Normal (a), lordotic (b, d) and highly deformed (c) Sparus aurata larvae observed under a stereomicroscope 1 day post-hatching (a) and (b), 4 days post-hatching (c) and 28 days post-hatching (d). VS, vitelline sac. Magnification × 17.
external feeding between days 2 and 5. Daily mortality increased from this period to day 20, then progressively decreased. By day 30, the percentage of larvae surviving was 29.4; this figure had decreased to 23.9% 2 months later, when larvae measured 7.5–15 mm length.

The most frequently observed deformations in the hatchery were axial deviations, operculum atrophies and cranial abnormalities. Twenty-seven percent of the newly hatched larvae observed displayed clearly distinguishable skeletal deformities (Fig. 3(b) and (c)). In these early larval stages, lordosis was a minor defect compared with the many other, more severe axial deviations observed (Fig. 3(c)). At this time, larvae lacked a vertebral column and the notochord was the only axial skeletal structure.

Larvae developed a mouth and the digestive tube (days 3–6 after hatching) and yolk sac reabsorption occurred. Larvae became active and commenced feeding. The number of deformed larvae progressively decreased from this period and, at the end of the larval period, they represented about 5% of surviving larvae. Most of these deformed animals were lordotic and many had curvatures up to 45° (Fig. 3(d)).

Lordotic specimens showed unbalanced growth, which started to be clearly noticeable 2 weeks after hatching. The difference progressively increased and, 150 days after hatching, lordotic juveniles measured 59 mm in length, while normal animals measured 64 mm.

Fig. 4. (a) Semithin sagittal section through the curvature region of a 1 day post-hatching lordotic larva stained with toluidine blue. Magnification ×150. (b) A connective septum penetrating the notochord (N). The perinotochordal tissue appears to be surrounded by thick connective tissue. Magnification ×600.
Under the microscope, sectioned lordotic larvae (Fig. 4(a)) showed a defective notochord. It was interrupted by septa composed of loose connective tissue and an inner thick layer, in continuity with the outer notochordal sheet, was conspicuous (Fig. 4(b)).

4. Discussion

When larvae are reared in hatchery conditions, the main objective for the aquaculturist is to obtain maximal growth and survival rates by the application of optimal conditions. However, these optimal conditions, along with the lack of predators and high food density, eliminate the natural selection that would occur in the natural environment. Therefore, the appearance of a high number of abnormalities at these conditions is not unexpected.

4.1. Lordosis and swimbladder non-inflation

It has been reported that the inability to inflate the swimbladder during post-larval growth leads to the development of spinal curvatures in fish (Kitajima et al., 1977; Kitajima, 1978; Paperna, 1978; Iseda et al., 1979; Kitajima et al., 1981; Chatain, 1994; Kitajima et al., 1994). Chatain and Ounais-Guschemann (1990) and Chatain (1994) reported that, after extending an oily layer on the surface of culture tanks, 90% of sea bream larvae were unable to inflate the swimbladder and developed lordosis. Juvenile fish with uninflated swimbladders were unable to stay in the water column because of their negative buoyancy, and displayed aberrant swimming behaviour. These authors suggested that this behaviour may provoke deformation of the axial skeleton (Kitajima, 1978; Chatain, 1982).

First inflation of the swimbladder occurs around day 8 after hatching. Our results showed that deformed larvae are present before this time. Thus, in addition to non-inflation of the swimbladder, other causes during embryonic development may be implicated in the appearance of lordosis and other abnormalities. However, our results showed that all lordotic juveniles lacked an inflated swimbladder. It is possible that lordotic larvae could have an impaired ability to inflate the swimbladder at the appropriate time. In this case, non-inflation of the swimbladder would be a consequence instead of a cause of lordosis. Moreover, if the main cause of lordosis were non-inflation of the swimbladder, an increase in the percentage of lordotic specimens after day 8 post-hatching (inflation period) would be expected. Our results did not show such an increase. Thus, in our study, most deformed fish appeared to be the result of defective embryonic development. On the other hand, a functional swimbladder was always present in adult fish whether lordotic or normal. Thus, it is likely that a delay in the development of the swimbladder occurs in lordotic sea bream. Late inflation of the swimbladder has been previously reported for Japanese sea bream (Chrysophrys major) (Kitajima, 1978; Chatain, 1982). In contrast to these studies, late inflation in S. aurata did not correct lordosis. Histological studies on uninflated swimbladders from lordotic juvenile Dicentrarchus labrax and S. aurata have shown the absence of a pneumatic channel and hypertrophy of swimbladder tissues (Giavenni and Doimi, 1983; Weppe and Bonami,
Therefore, late inflation of the swimbladder in lordotic specimens should occur by activation of the gas gland.

4.2. Lordosis in larvae

Although 27% of newly hatched \textit{S. aurata} larvae showed axial deformities, only some were lordotic. The percentage of deformed larvae, mostly lordotic, decreased to 5 at 60 days. These results indicate: (1) as expected, mortality of deformed larvae exceeded that of normal larvae, and (2) survival of lordotic larvae was higher than that of other deformed larvae. It is therefore likely that, in contrast to other grosser deformities, lordosis in larvae does not totally interfere with swimming and feeding behaviour and is not deleterious.

Most of the juvenile lordotic specimens observed probably developed from surviving lordotic larvae. If so, the primary causes of lordosis emanated from defects in axial structures during embryonic development. Therefore, congenital lordosis could be due to: (1) genetic causes; (2) composition of the yolk; (3) environmental causes such as light, temperature, mechanical shock, pollutants, infections, etc., that could affect parentals or embryos. Skeletal deformities have been experimentally induced by genetic manipulation (Wolfarth et al., 1961; Kincaid, 1976; Paperna, 1978; Piron, 1978). Increasing numbers of genes have been found to be related to the correct development of an axial skeleton in fish (cf. Westerfield, 1994). Pathogens such as the myxosporidian \textit{Myxosoma} sp. (Treasurer, 1992) may induce congenital or postnatal skeletal deformations in fish. The possible role of environmental (Haya, 1989; Weis and Weis, 1989; Wiegand et al., 1989; Caris and Rice, 1990) and nutritional (Walton et al., 1984; Akiyama et al., 1989; Hinton et al., 1992) factors in the aetiology of spinal deviations in fish has been extensively discussed.

During embryonic and early larval development, axial deviations result from defective development of the notochord and perinotochordal connective sheet (Santamaría et al., 1994). This in turn could lead to further malformations in the vertebral column, e.g. lordosis. Alteration of collagen metabolism has been reported to be related to notochordal malformation in \textit{Xenopus laevis} tadpoles (Schultz et al., 1985; Riggin and Schultz, 1986). The mechanism of notochord malformation during embryonic development is still not clear.

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References


