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Deformities in larval gilthead sea bream (*Sparus aurata*): A qualitative and quantitative analysis using geometric morphometrics

Yves Verhaegen [a], Dominique Adriaens [a,*], Tania De Wolf [b], Philippe Dhert [c], Patrick Sorgeloos [d]

[a] *Evolutionary Morphology of Vertebrates, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium*
[b] *Maricoltura di Rosignano Solvay, srl, Via P. Gigli (Loc Lillatro), I-57013 Rosignano Solvay, Italy*
[c] *INVE Technologies, NV, Oeverstraat 7, B-9200 Baasrode, Belgium*
[d] *Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Rozier 44, B-9000 Gent, Belgium*

Abstract

Deformities in commercially raised fish are a common source of downgrading of product value. During the intensive rearing of gilthead sea bream (*Sparus aurata*), opercular deformities are the most commonly observed type of deformation (affecting up to 80% of the fisheries stock), sometimes showing a severe inward folding of the operculum. They are non-lethal malformations that appear during the larval stage but affect growth rate and morphology, with a significant economic loss as a consequence. In order to exploratory quantify and qualify these deformities, geometric morphometric analyses were performed on the external morphology from larvae with an age ranging from 50 to 69 days post-hatching (DPH), and on the cranial skeleton of 110 DPH old juveniles. The results showed several osteological cranial shifts and a striking left–right independency associated with deoperculation. Even though a significant size difference was observed at 65 DPH between normal and deoperculated specimens, allometries during the examined growth stages still appear to be very similar in normal and deoperculated specimens. At 69 DPH deoperculated specimens differed significantly from the normal specimens in their external morphology based on its shape variables, but the results suggest that discrimination is possible from earlier stages. Further analyses are needed, but the usefulness of this approach towards developing an early detection tool could be demonstrated.

Keywords: *Sparus aurata*; Opercular complex; Abnormality; Geometric morphometrics; Ontogeny

1. Introduction

Gilthead sea bream (*Sparus aurata*), being one of the first intensively cultivated species in the Mediterranean (Fishery Information, Data and Statistics Unit, Food and Agriculture Organization of the United Nations (FAO), 2005), has become an important research topic over the years (Koumoundouros et al., 1997a). The focus has mainly been on aquacultural aspects, and the implementation of techniques to improve reproductive success, survival and growth (Tandler and Helps, 1985; Polo et al., 1991; Mourente et al., 1993; Parra and Yufera, 2000; Papandroulakis et al., 2002; Sadek et al., 2004). As a maximal yield in growth and reproductive success may come into reach, problems arise with respect to the overall quality (especially deformations) of the fry and subsequent juvenile fish. Although some studies on larval quality have been performed in the past (*e.g.* Paperna,

* Corresponding author. Tel.: +32 9 264 52 19; fax: +32 9 264 53 44.
E-mail address: dominique.adriaens@ugent.be (D. Adriaens).

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1978; Koumoundouros et al., 1995; Furuita et al., 2000; Boglione et al., 2001; Cahu et al., 2003), future research efforts may need to concentrate even more on a qualitative rather than quantitative improvement of the produced fish, especially in order to expose the nature behind developmental and growth problems frequently experienced in cultivating facilities. Some examples of common abnormalities in cultivated fish are axial deformities, whether or not related to swimbladder abnormalities (such as saddle-back syndrome and axial lordosis) (*e.g.* Alfonso et al., 2000, Boglione et al., 2001), abnormal caudal fin development (*e.g.* Koumoundouros et al., 1997b) and opercular deformations. Especially the distorted development of the operculum in *S. aurata*, as well as in some other cultivated species, have been a source of product downgrading and substantial economic losses over many years (affecting up to 80% of farmed gilthead sea bream) (Paperna, 1978; Francescon et al., 1988; Chatain, 1994; Andrades et al., 1996).

In order to improve cost-efficiency for farmed market-sized gilthead sea bream, a fast and early recognition of developing abnormalities is of utmost importance for fish farmers, as well as to unravel the effect of rearing parameters in order to minimize the prevalence of these deformities a priori. As a result, research efforts have been focusing on biometric screening of larvae for such deformities (Koumoundouros et al., 1997a). However, studying the pattern behind deformities and related growth disturbances is frequently based on biometric analysis, relying on point-to-point measurements of several structures and parts of the body plan (Koumoundouros et al., 1997a; Lindesjöo et al., 1994). Such an approach is useful for studying size changes, thus growth, but is insufficient in describing aspects of shape adequately (especially size-independent shape, even when using ratios) (see Richtsmeier et al., 2002; Dodson, 1978), thus possibly problematic when studying deformities. For that reason, geometric morphometrics allow a more complete description of shape and thus a more robust and detailed analysis of shape variation in growing and transforming fishes (Rohlf and Marcus, 1993; Adams et al., 2004). This approach has proven to be useful for studying shape changes of commercially important fishes in the past (*e.g.* Cavalcanti et al., 1999; Koumoundouros et al., 2005; Loy et al., 1998; 2000; 2001; Valentin et al., 2002), including *S. aurata* (Loy et al., 1999). All these shape analyses focused on populations in the wild, or the effect of rearing conditions on the overall body shape in adult specimens.

We performed a landmark-based geometric morphometric analysis on both external and internal (skeletal) morphology of different age groups of *S. aurata*. The specific goals of this exploratory study are: (1) to analyze quantitatively and qualitatively the nature of osteological aberrations in the skull associated with deoperculation in 110 day old juveniles, and (2) to verify to what degree shape changes in the external morphology (total body shape) co-occur with opercular deformities between 50 and 69 days post-hatching. Such an approach will assist in future efforts to allow a fast recognition protocol for detecting early opercular deformation in cultivated gilthead sea bream.

2. Material and methods

2.1. Rearing conditions

All specimens originate from the commercial sea bream hatchery Maricoltura di Rosignano Solvay (Italy). Eggs were obtained from a mixed broodstock of reared and locally wild caught adults. The broodstock was fed a mixed diet of Lansy Breed® (INVE Aquaculture NV, Belgium) pellets and fresh seafood (shrimps, mussels, crabs and fish). Spawning occurred under regulated light and temperature conditions in 2800 l tanks. The collected eggs hatched in a 140 l incubation tank approximately 48 h after spawning and were transferred indoors to 6000 l larval rearing tanks after 24 h. Initial rearing density was approximately 150 larvae/l. Larvae were raised under a semi-closed circuit of filtered natural seawater (39% salinity) originating from the nearby coast. Temperatures and dissolved oxygen concentrations were continuously kept closely to 19 °C and between 6–12 ppm, respectively. The following light intensity regime were applied: 0–1 DPH at 80 lx, 2–3 DPH at 250, 4–13 DPH at 400, 14–39 DPH at 250 lx and subsequent weaning at natural shaded sunlight (4000 lx). High aeration during 0–1 DPH kept passively moving larvae suspended, with aeration at low level during green water phase (sufficient to distribute algae over water column). After this phase passive aeration induced by water flow was sufficient. The creation of water currents was minimized through the vertical position of the water inlet just above the water surface to allow a natural swimming behaviour of the developing fish. As from 48 DPH, the post-metamorphic phenomenon of schooling was prevented by placing a vertically submerged net in the circular tanks.

Surface skimmers were used to inhibit lipid film formation on the water surface (Chatain and Ounais-Guschemann, 1990). The ‘green water’ technique was applied during the period 3–28 DPH, using two phytoplankton strains (*Nannochloropsis sp.* and *Isochrysis sp.*). The larvae were fed live feed starting on day three