VISUALIZING MINERALIZATION IN DEFORMED OPERCULAR BONES OF LARVAL GILTHEAD SEA BREAM (*SPARUS AURATA*)

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Mineralisation in deformed opercles
Summary

During the rearing process of gilthead sea bream (Sparus aurata), abnormal development of the opercular bone is particularly common (Koumoundouros et al. 1997). In order to alleviate its occurrence in rearing facilities, it’s crucial to identify the very first physical signs of deviation in normal skeletal development. Nano-CT-scanning was tested for its applicability to quantify deviations in bone mineralization levels. Seven opercles were dissected from larvi of 65 days post hatching, randomly sampled at the commercial sea bream hatchery Maricoltura di Rosignano Solvay (Livorno, Italy). The samples were nano-CT-scanned and computationally reconstructed. Mineralization intensity was colorcoded using Amira software, resulting in a detailed visualization of opercular morphology and mineralization patterns. In conclusion, nano-CT-scanning promises to be a good tool to both describe morphology and detect mineralization levels in the early onset of deformities.
Introduction

After 20 years of successful aquaculture research, skeletal deformities remain a major cutback in fish production worldwide (Fraser & Nys, 2005). During the rearing process of gilthead sea bream (Sparus aurata), being the most intensively cultivated fish species in the Mediterranean region, abnormal development of the opercular bone is particularly common (Koumoundouros et al. 1997). Affected individuals show an inward folding of the operculum (fig. 1a) and significantly diminished growth rates, consequently downgrading the marketing image of the product (Beraldo et al. 2003). As the abnormality develops at an early larval stage (17 days post hatching (DPH) and most probably earlier, Galeotti et al. 2000), identifying physical criteria that allow detection of the very first deviations in normal skeletal development are crucial to alleviate its occurrence in rearing facilities. One of potentially informative criteria is the level of hydroxyl-apatite mineralization of the opercula. In order to explore this potential, two different tools are tested for their applicability to characterize those criteria: (1) nano-CT scanning and (2) histological stainings (Masson’s trichrome and toluidine blue). Here, the results of the nano-CT scanning will be discussed.

Materials and methods

A total of 1141 specimens of gilthead sea bream (Sparus aurata) were collected from a commercial hatchery (Maricoltura di Rosignano Solvay), and fixed in a 4% NaH₂PO₄–Na₂HPO₄ buffered formalin. We selected a range of phenotypically normal to severely deformed S. aurata larvi (65 DPH, average standard length (LS) 16.8 ± 1.5 mm, from Maricoltura di Rosignano Solvay, Italy). Although the deformity is already visible at 17 DPH (Galeotti et al. 2000), we started the research with the analysis of 65 DPH old specimens. At 65 DPH, the deformity is clearly visible with the naked eye, so that an unambiguous CT-image can be acquired and serve as a frame of reference. In a next phase, younger specimens will be analyzed and compared with the frame of reference. Seven opercles were dissected, dehydrated, mounted on gum and subjected to a nano-CT-scan (resolution of the CT-sections were approximately 1/1000 of the longest axis of the bone).
CT-scanning was performed at the UGent CT-facility (www.ugct.ugent.be) (Masschaele et al., 2007). The specimens were scanned using a directional tube head, using an a:Si flat panel (Varian Paxscan 2520) detector with CsI scintillator. The raw data were processed and reconstructed using the in-house developed CT software Octopus (Vlassenbroeck et al., 2007) and rendered with Amira 4.1 (Mercury Systems).

To visualize levels of mineralization densities, the contrast level of the CT-images was optimized to remove voxel information from soft tissue. Colour gradients were applied to visualize the different levels of mineralization densities in the bone tissue (fig. 1d-f). Four parts of the opercle were used for comparison, along a horizontal plane (fig. 1i-m): at the level of the distal process of the opercle (fig. 1j), at the centre point of the opercular articulation (fig. 1k), at midway of the ventral plate of the opercle (fig. 1l), and at the lowest quartile of the ventral plate of the opercle (fig. 1m). Apart from mineralization densities, also differences in level of trabeculation of the bone tissue were compared.

Results and discussion

The ossification pattern of a phenotypically normal opercle at 65 DPH shows a rather well developed opercular bone, comprising a plate-like ventral part with solid bone and rather homogenous mineralization levels (fig. 1j-m). At the mid-region of this ventral plate, the caudal rim can show some level of outward folding, whereas the anterior rim is more densely mineralized. Additionally, the anterior rim shows medially directed trabecular projections that are completely lacking caudally. At the level of the articulatory facet, trabeculation is the most extensive, with a horizontal crest (at the medial face of the bone) running caudally up to the distal tip. Dorsal to this crest, the opercular bone is plate-like with a rather homogenous distribution of minerals.
After colour gradient application, the volume-rendered 3D models indicate a denser mineralization in deformed opercles (overall more brightly coloured, fig. 1f, h) than in normal opercles (fig. 1e, g). This increased mineralization could be induced by an osteogenic response to increased mechanical loading of the opercle, as a result of this folding. In order to find out what occurs first: the aberrant mineralization or the folding of the opercle, larger samples of younger ontogenetic stadia will be scanned.

**Conclusion**

Focusing on traits that characterize bone and ossification may yield a powerful tool to detect early onset of deoperculation, and thus allow to test the effect of different rearing conditions on its prevalence. Several of these traits can be suggested for monitoring, such as bone shape, collagen content or mineralization content. In this study, we tested the applicability of nano-CT-scanning with graphical 3D-volume rendering as a tool to detect aberrant patterns of mineralization in bone as a proxy for early onset of opercular deformation. It provides a detailed 3D image of the gill cover morphology and enables visualization of differences in mineral density. The obtained results indicate that performing quantitative analysis on different levels of mineralization, using nano-CT-scanning, is feasible and may allow statistical testing for effects of specific rearing conditions that are targeted for in order to reduce the occurrence of opercular deformations. Once the earliest signs of deviation from normal skeletal development are pinpointed, the obtained histological characters can allow us to correlate body shape characters with opercular deformations at the earliest stages.

Verhaegen et al. (2007) could significantly separate deoperculated from normal specimens at 69 DPH, based on small differences of the external morphology. Body shape is a potential screening criterion to be applied in hatcheries.
References


Figure legends

**Fig. 1:** (a) Habitus of 65 DPH old larva with deformed right opercle; (b) normal phenotype of dissected opercle; (c) phenotype of highly deformed opercle; (d) reconstruction of nano CT-scanned 65 DPH larva with deformed right opercle with colourmap threshold, ranging from low (red) to high (white) electron density (mineralization); (e-f) volume renderings of two gill covers, visualizing mineral density by same colour mapping of normal gill cover (e) and severely deformed gill cover (more densely mineralised) (f); (g-h) axial sections through the opercles as indicated by the blue lines, show highly bended regions being more densely mineralized; (i-m) phenotypically normal opercle of 65 DPH old larva (reconstruction from CT-data) (i), with mineralization density showed in horizontal sections at four levels (j, k, l, m).