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SEMI-QUANTITATIVE SCALES FOR THE ASSESSMENT OF THE SEVERITY AND GENETIC PARAMETERS OF SKELETAL ABNORMALITIES IN SEABREAM LARVAE

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Introduction

Skeletal abnormalities develop mostly during the early ontogenetic stages and have serious economical impacts to finfish aquaculture, notwithstanding the numerous studies to prevent their development. Jaw abnormalities such as pugheadness, crossbite, reduction or elongation of the lower jaw, develop frequently in reared fish and have significant effects on both morphology and performance (reviewed by Koumoundouros 2010; Boglione et al., 2013). The genetic basis of jaw abnormalities has been examined for a variety of fish species, after a binary phenotypic categorization of juvenile or adult fish (e.g. García-Celdrán et al., 2016; Nguyen et al., 2016; Sawayama and Takagi,2016).

In the present study we developed a set of semi-quantitative scales for the early assessment of jaw abnormalities in Gilthead seabream larvae. Developed scales were used to estimate the heritability of various jaw deformities at the end of the larval rearing phase (39 days post-hatching, dph).

Materials and methods

A random sample of 1400 seabream larvae (39dph) were taken from a mass spawning of 64 males and 63 females, which were reared according to the standard methodology of the commercial hatchery. Samples were anaesthetized, fixed in 5% phosphate-buffered formalin and stained for bone and cartilage. All specimens were examined for the presence of abnormalities in the maxillaries (Ma), pre-maxillaries (Pm) and rostral cartilage (Rc) (Fig. 1A). Each abnormality was scored individually, leading to a matrix that contained all different abnormalities. Scoring of paired elements was performed for both sides of the body. After their phenotypic examination, all the specimens were subjected to DNA extraction which was done with a commercial kit and the samples were subjected to PCR for 9 microsatellites for parentage analysis. Variance components were estimated with REML using an animal model.

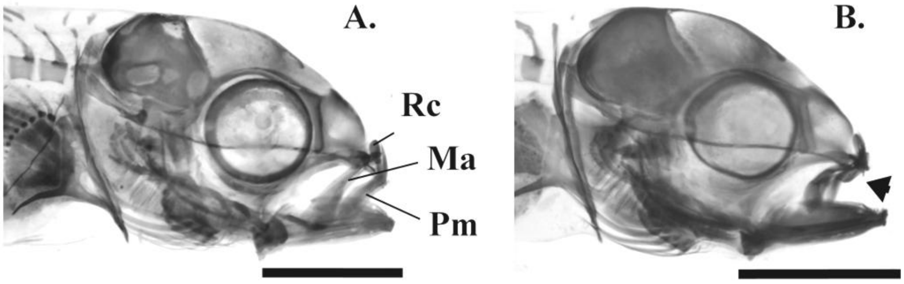

Fig 1. Normal (A) and abnormal (B) anatomy of the upper jaw in seabream larvae. Rc, rostral cartilage. Ma, maxillary bone. Pm, premaxillary bone. Scale bars are equal to 0.5mm.

Results and discussion

The most frequent abnormalities were the narrowing (n-Ma, 89.2±0.1%) and the inward bending of the maxillaries (b-Ma, 25.6±3.4%), the fusion of maxillaries with pre-maxillaries (f-Ma/Pm, 9.5±0.6%) and the partial to complete lack of the premaxillaries (a-Pm, 8.7±0.3%) (Fig. 2). Other minor abnormalities (ranged between 0.8-3.2%) were the partial to complete lack of the maxillaries (a-Ma), the dorso-ventral curvature of maxillaries and pre-maxillaries (d-Ma and d-Pm, respectively) and the lack of the rostral cartilage (a-Rc). After the calculation of the new indices, the examined larvae were placed on a scale with 2 to 13 levels based on the severity of the abnormalities (depending on the index, Table I). Genetic analysis revealed that the fusion of maxillaries with pre-maxillaries, and the narrowing of maxillaries presented significant heritabilities (0.23±0.06 and 0.13±0.04 respectively, Table II).

To our knowledge it is the first time where both qualitative and quantitative evaluation of jaw abnormalities took place in seabream larvae, allowing us to fully characterize the phenotypic range of abnormalities during the phase of their development. Compared with the macroscopic scoring of abnormalities during the juvenile or adult stages, the microscopic analysis of whole-mount stained individuals is characterized by higher anatomic precision, potentially allowing for the distinction between different abnormality types.

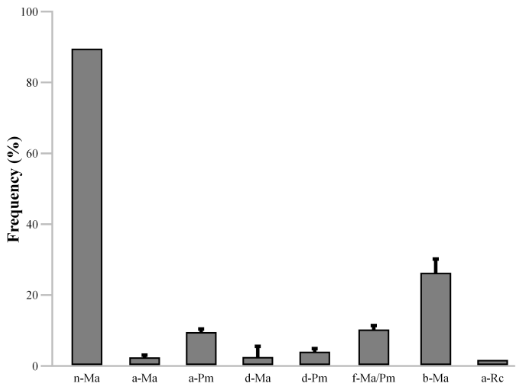

Fig. 2. Frequency of the different abnormalities affecting the skeletal elements of the upper jaws. n-Ma, narrowing of maxillaries. a-Ma, a-Pm, partial to complete lack of maxillaries and premaxillaries respectively. d-Ma, d-Pm, dorso-ventral curvature of maxillaries or premaxillaries respectively. f-Ma/Pm, fusion between maxillaries and premaxillaries. b-Ma, inwards bending of maxillaries. a-RC, lacking of rostral cartilage.

Table I. Mean, SD, range and size of the calculated abnormality scales. For each bilateral abnormality, the sum of the scores of the two sides was calculated. All abnormalities were scored for a total of 1254 larvae. The mean indices are proportional to the severity of the abnormalities.

Character	Mean	SD	min	max	Scale size (levels)
n-Ma	2.777	1.452	0	6	7
a-Ma + a-Pm	0.508	1.500	0	12	13
d-Ma	0.067	0.378	0	3	3
d-Pm	0.109	0.484	0	4.5	4
f-Ma/Pm	0.438	1.193	0	6	5
b-Ma	0.813	1.292	0	6	5
a-Rc	0.050	0.385	0	3	2

Table II. Estimates (±SE) of heritabilities (h^2) for the different anatomical abnormalities.

Abnormality	Mean h^2±SE
n-Ma	0.13±0.04
a-Ma + a-Pm	0.02±0.02
d-Ma	0.01±0.02
d-Pm	0±0.02
f-Ma/Pm	0.23±0.06
b-Ma	0.06±0.03
a-Rc	0±0.02

Acknowledgements

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