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The use of texture analysis in the vertebral morphometric study of a lordotic chub (*Squalius cephalus* L.)

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ABSTRACT

Objective: To characterise vertebral aspects of a lordotic chub (*Squalius cephalus* L.) using texture analysis by radiograph and to verify possible alterations in the vertebral microarchitecture of the lordotic tract.
Methods: Four vertebrae belonging to the lordotic tract were analyzed for their texture features by means of a software package (MaZda vers. 4.6) and were compared, for the same features, to other four vertebrae cranial (anterior) to the lordotic vertebrae.
Results: Two representative features were selected out of 259 extracted texture features by means of the convex hull selection method. In particular, (5, 5) sum of squares (according to co–occurrence matrix method) and 45° short run emphasis (according to run–length matrix method) were used to classify, without classification errors, two selected spinal tracts (the lordotic one and the immediately cranial one, both corresponding to the anal fin pterigiophores vertebral portion) according to the following methods: linear discriminant analysis and hierarchical clustering.
Conclusions: This is the first known application of texture analysis in medical imaging applied to fish species and represents a start point for further studies in aquariology and in aquaculture, where spinal deformities occur relative frequently and are cause of economic losses.

1. Introduction

To date, there is not a standardized definition of image texture, though the latter is naturally and subjectively distinguished by human vision as smoothness, coarseness, *etc.*[1–3]. Texture may contain substantial information about the structure of any physical object and its graphical representation; therefore, it is a pivotal parameter in image analysis[4–6]. Referring to biomedical science, texture analysis has been successfully used in medical imaging, diagnostic histopathology and tissue morpho–functional studies[4–11].

The main object of the present case study was to detail the texture parameters of the vertebral lordotic tract in a European chub [*Squalius cephalus* L. (*S. cephalus*)], and to compare it, for the same features, to the tract immediately cranial (anterior) to the previous. Both vertebral tracts were related to the anal fin pterygiophores. The same European chub has been already studied from a morphometric and radiographic point of view, in order to verify the possible involvement of alteration in vertebral bone microarchitecture, as previously hypothesized, and to improve the knowledge about lordosis etiopathogenesis in fish[12].

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2. Materials and methods

The chub (*S. cephalus* L.) was captured in the wild at age class 0+ (standard length: 6 cm) and was hosted in a 50 L glass aquarium until its death at age class IV (standard length: 11.9 cm; body mass: 31 g; condition factor: 1.32). After a year from the introduction, the chub developed an initial mild ventral vertebral curvature affecting the caudal tract. Lordosis progressed more and more and, as death occurred, the caudal tract of the fish was approximately perpendicular with respect to the trunk. Further morphometric and radiographic data are available in study of Manera M *et al*[12].

Previously obtained radiographs were selected, scanned and saved as TIFF gray level uncompressed file format. The four vertebrae belonging to the lordotic tract (30, 31, 32, 33) were analyzed for their texture features by means of a public domain software package for image texture analysis (MaZda vers. 4.6, Szczypinski P)[4-6,13], and were compared, for the same features, to other four vertebrae immediately cranial (anterior) to the lordotic vertebrae. All the analyzed vertebrae were related to the anal fin pterygiophores (namely they all belonged to same vertebral region), to ensure the same normal bone microarchitecture (Figure 1). In particular, regions of interests (ROIs) were selected by proper polygonal selection instrument of MaZda through the vertices of the fish typical clepsydra-shaped vertebrae, in latero-lateral projection.

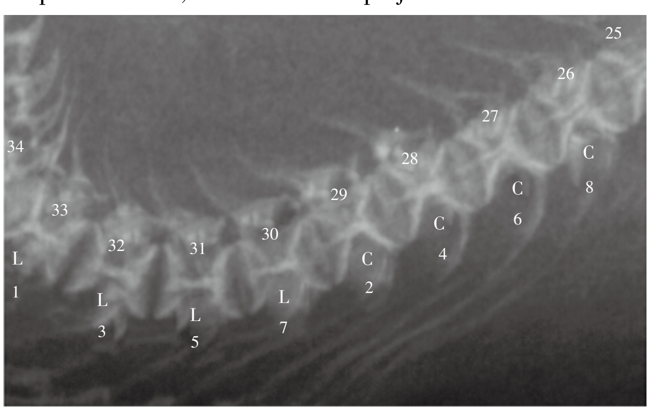

Figure 1. European chub.
Radiograph of the caudal vertebral tract corresponding to anal fin pterigiophores clearly appreciable beneath vertebral bodies. Vertebrae are numbered above the vertebral body in cranio-caudal versus. Numeric identification of vertebrae, as adopted in the analysis, is reported below the vertebral body, according to vertebral tract (L: lordotic; C: control).

Texture features from image histogram, image gradient (8 bits/pixel), run-length matrix (8 bits/pixel), co-occurrence matrix (8 bits/pixel; 1, 2, 3, 4, 5 bits distance), autoregressive model were computed. Normalisation (±3 sigma) was adopted for the computation of all the above mentioned features. The extracted features list was histogram (total features=9), mean, variance, skewness, kurtosis, 1% percentile, 10% percentile, 50% percentile, 90% percentile, 99% percentile; gradient (total features=5), absolute gradient mean, absolute gradient variance, absolute gradient skewness, absolute gradient kurtosis, percentage of pixels with nonzero gradient; run length matrix (four directions: 0°, 45°, 90°, 135°; total features=20), run length nonuniformity, gray level nonuniformity, long run emphasis, short run emphasis, fraction of image in runs; co-occurrence matrix (five between-pixels distances: 1, 2, 3, 4, 5; four directions: 0°, 45°, 90°, 135°; total features=220), angular second moment, contrast, correlation, sum of squares, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference variance, difference entropy; autoregressive model (total features=5), parameter θ_1, parameter θ_2, parameter θ_3, parametr θ_4, parameter σ. Cumulatively, a total of 259 texture features were computed for each of the analysed vertebrae. Further details about texture parameters are available in MaZda technical documentation[4,14].

Thereafter, the most representative features were selected by convex border (hull) features selection/reduction method[4,14]. Convex hull is *per se* a powerful discriminative method and provides a classification rule to separate classes. Actually, as stated by Szczypinski *et al*.[4], convex hull "is essentially a discriminant analysis method of supervised learning for reduction of vectors dimensionality and for data classification".

Obtained numerical data were assessed for normality by Shapiro-Wilk test and the following classification analyses were performed on them: linear discriminant analysis and hierarchical cluster analysis (Euclidean distance, between groups average linkage, Z scores). SPSS® 14.0.2 (SPSS Inc., Chicago, IL, USA) was the statistical package for data analysis.

3. Results

Radiographic appearance of the lordotic vertebral tract is reported in Figure 1. Convex border (hull) feature selection method resulted in the following classification rules: Class 1 (vertebrae of the lordotic tract), 0.917≥45° short run emphasis≥0.905; Class 2 (vertebrae of the normal tract), 1 923.463≥(5, 5) sum of squares≥1 824.131. Means and standard errors of mean of the two selected features, according to vertebral tract were: 45° short run emphasis, (0.912±0.001) (lordotic), (0.922±0.001) (normal), 0.917±0.002 (total); (5, 5) sum of squares, (lordotic) (1 742.699±24.93), (1 870.613±5.064) (normal), (1 806.656±26.889) (total). Linear discriminant analysis (with both original and cross-validated data) permitted the clear discrimination of the two vertebral tracts without any classification error. Results of hierarchical cluster analysis are reported in Figure 2. Separation between the two vertebral tracts is clearly appreciable. Furthermore, the lack of homogeneity in Euclidean distances, thus in texture parameters and, possibly, in bone microarchitecture, is evident for the vertebrae belonging to the lordotic tract.

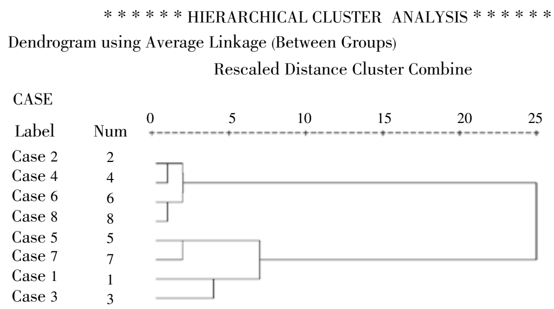

Figure 2. Hierarchical cluster analysis dendrogram.
Clear separation between vertebral tracts is evident. Uneven numbers refer to lordotic vertebrae, whereas even numbers refer to control/normal vertebrae as reported in Figure 1.

4. Discussion

Texture features analysis provides a powerful classification/discrimination method of vertebrae according to the two vertebral tracts and supports what previously suspected with regard to the alteration of bone microarchitecture in the vertebrae belonging to the lordotic tract[12]. Actually, texture analysis has been successfully applied on radiographic images to study bone microarchitecture alterations and results comparable with those of histomorphometric and densitometric methods have been obtained[15,16].

Results of present survey contribute to increase the knowledge about etiopathogenesis in the specific case report with particular regard to possible nutritional imbalances (energy to protein ratio and both macro- and micro-constituents)[12,17]. Moreover, present case study is the first known application of texture analysis in medical imaging applied to fish species and represents a start point for further studies in aquariology and particularly in aquaculture where spinal deformities occur relative frequently and are cause of economic losses[17,18].

Conflict of interest statement

We declare that we have no conflict of interest.

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