



-RESEARCH ARTICLE-

Age determination with alcian blue staining technique for *Lagocephalus sceleratus* in Mersin Bay, Turkey, northeastern Mediterranean

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Abstract

In this study, alcian blue staining technique was used to enhance the visibility of the band on vertebrae of *Lagocephalus sceleratus* from Mersin Bay, Turkey, Northeastern Mediterranean. A total of 77 *L. sceleratus* samples were captured by commercial purse seine nets at depths between 24 and 50 m from Mersin Bay (Turkey). The total length ranged from 8.9 to 78.4 cm and body weight varied between 7.59-4750 g for *L. sceleratus*. Age determinations were carried out using whole vertebrae. A total of 4 vertebrae from each specimen were read by two independent readers. All images were taken using a Leica S8APO brand microscope with a high resolution Leica Application suit (Las V4.8) software. Vertebral images were then enhanced using Adobe Photoshop CS2 to improve sharpness and clarity. Of all the 77 staining vertebrae were readable and aged by both readers, resulting in an IAPE of 7.8 %. The maximum age was found as 10+ years old.

Keywords:

Alcian blue staining, growth ring, Silver-cheeked toadfish, *Lagocephalus sceleratus*, northeastern Mediterranean Sea.

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Introduction

The Suez puffer fish (*Lagocephalus suezensis* (Clark & Gohar 1953)) is one of the Lessepsian fish species inhabiting eastern Mediterranean Sea and originally endemic to the Red Sea. This species

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entered to the Mediterranean from the Red Sea via the Suez Canal. It feeds on small benthic invertebrates, spawns in the summer season. Eggs and larvae are planktonic, when threatened, The Suez puffer fish is capable of inflating its body by rapidly engulfing water or air. (Golani et al., 2006). First record of this species in Mediterranean was made in 1977 from the Lebanon coast (Mouneimne, 1977). Its distribution expanded to other areas of the Mediterranean in time (Israeli coast: Golani (1996), Turkish coast: Bilecenoglu et al. (2002), Syrian coast: Saad (2005), Rhodes Island: Corsini et al. (2005), Libyan coast: Ben-Abdallah (2011)). Length-weight relationships, some growth, reproduction and diet characteristics of this pufferfish were studied by Aydin (2011) and Başusta et al. (2013) in the Mediterranean coasts of Turkey. But there is no information on the age pattern of this species in the region.

Numerous techniques have been used in the attempts to enhance the visibility of growth bands in vertebrae. The success of each technique is often species specific and slight modifications may enhance the results (Goldman et al., 2012). Alcian blue staining technique have been used to enhance the visibility of age bands on the vertebrae of *Rhinobatos rhinobatos* (Basusta et al., 2008) and *R. cemiculus* and *Myliobatis aquila* (Basusta et al., 2010). In this study, alcian blue staining technique was used to enhance the visibility of age bands on vertebrae of *Lagocephalus sceleratus* from Mersin Bay, Turkey, Northeastern Mediterranean.

Material and Methods

A total of 77 *L. sceleratus* samples were captured by commercial purse seine nets at depths of 24 and 50 m in Mersin Bay (Figure 1). The total length and body weight ranged from 8.9 to 78.4 cm and from 7.59 to 4750 g for *L. sceleratus*, respectively (Figure 2).

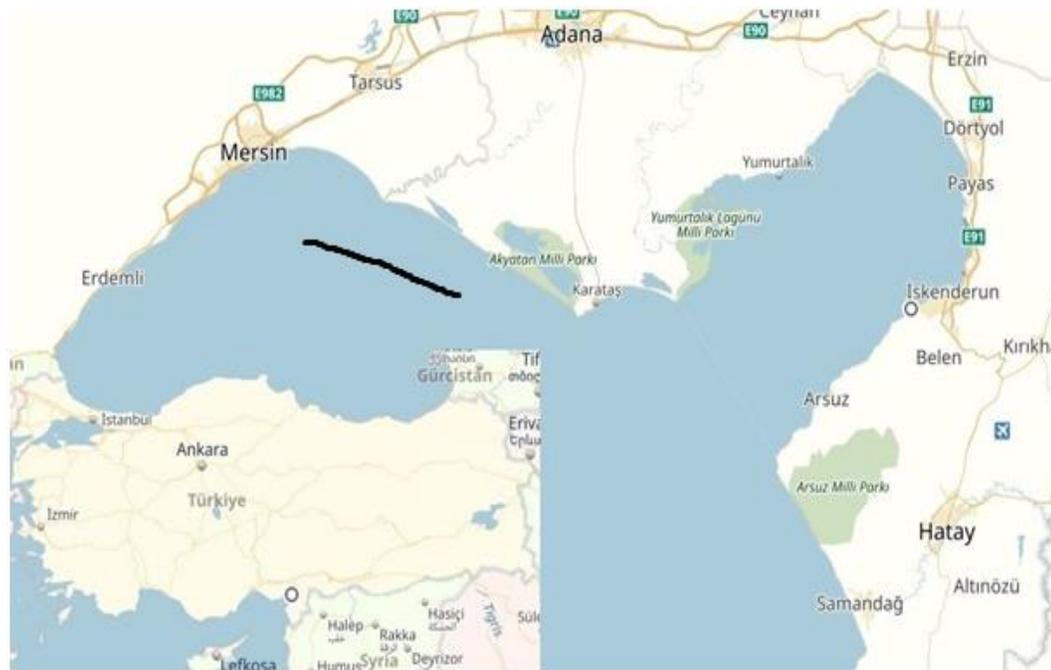


Figure 1. Map of sampling area in Mersin Bay, northeastern Mediterranean



Figure 2. Length measurement of *Lagocephalus sceleratus*

For the age determination in *L. sceleratus*, 8-10 vertebrae were removed from the widest portion of the body for each specimen. Remaining muscle tissue was removed from each centrum using a knife and the centra were soaked in 5% sodium hydrochloride for one day and then rinsed in distilled water for the removal of excess of connective tissue. The vertebrae were preserved in 70% ethanol until the examination.

In this study, alcian blue staining technique was used to enhance the visibility of the age bands on vertebrae. The centra was soaked in alcian blue solution (16 ml 100% ethanol, 2 mg alcian blue and 4 ml glacial acetic acid in 0.8 ml distilled water) for 12 h (Başusta et al., 2008).

A total of 4 vertebrae for each specimen were read independently by two readers. All images were taken using a Leica S8APO brand microscope with a high resolution Leica Application suit (Las V4.8) software. Vertebral images were then enhanced using Adobe Photoshop CS2 to improve sharpness and clarity. The index of the average percentage error (IAPE) was calculated to assess the precision of the age determinations between two independent readers. The equation (Beamish & Fournier (1981)) is expressed as follows:

$$IAPE_j = \frac{1}{N} \sum_{j=1}^N \left[\left(\frac{1}{R} \sum_{j=1}^R \frac{x_{ij} - x_j}{x_j} \right) \right] * 100\%$$

where N is the number of fish aged, R is the number of times each fish was aged, x_{ij} is the *ith* age determination of the *jth* fish, and x_j is the mean age calculated for the *jth* fish.

Results and Discussion

Alcian blue staining technique was used to enhance the visibility of the bands on vertebrae. Vertebrae of *L. sceleratus* were stained to visualize age bands (Figure 4). Vertebrae were readable in all of 77 specimens stained and aged by two independent readers. Resulting IAPE was 7.8 %. The maximum age was found as 10+ years old. Readability of the bands on vertebrae was highly enhanced using Alcian blue staining technique. In this technique, the percentage of readable vertebrae was found to be 84% for *Rhinobatos rhinobatos* (Başusta et al., 2008) and 90% for *R. cemiculus* and *Myliobatis aquila*. and it was 100%. for this study.

Thus it can be claimed that the alcian blue staining technique can be used successfully for the age determination of pufferfishes from vertebrae. As it was supported with previous studies the technique is valid for other species and can reliably used in a variety of vertebral organisms.

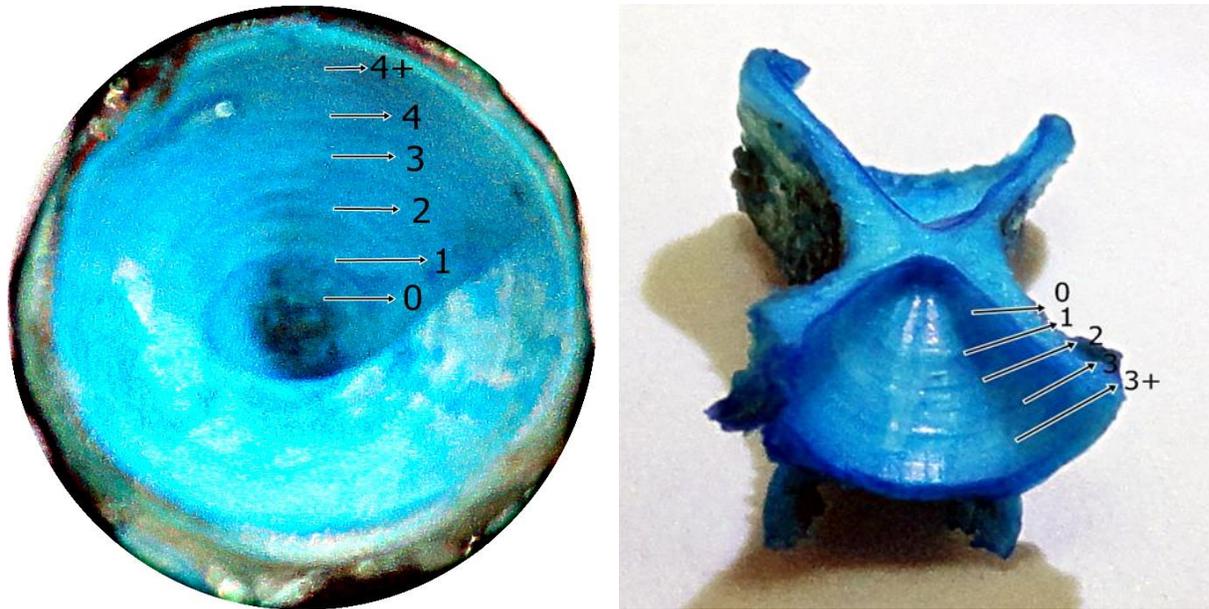


Figure 4. Occurrence of age bands following alcian blue staining for *Lagocephalus scleratus*

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