

## A STUDY ON THE ELABORATION OF A COLORATION PROTOCOL ON THREE SMALL-SIZE FRESHWATER FISH SPECIES: *CARASIVS GIBELIO* (BLOCH, 1782) *PSEUDORASBORA PARVA* (TEMMINCK & SCHLEGEL, 1846) AND *ALBURNUS ALBURNUS* (LINNAEUS, 1758) (PISCES, CYPRINIDAE), USEFUL IN AGE-DETERMINATION BY MEANS OF GROWTH RING COUNTING

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### INTRODUCTION

The problem of age determination in fish rises often throughout field studies focusing on freshwater fish populations or in aquaculture research interested in the growth of the individuals.

In this respect, one may choose specific methods of different efficiency or relevance, as well as of more or less invasive or damaging nature on the research material.

Considering that counting annual growth rings on the fish scales is often difficult and inexact, when following the existent procedures (extracting, mechanical cleaning, degreasing, polishing, observing by means of transparency) at small size species or at species having small size scales, we have focused on elaborating a procedure that will allow both efficiency in field research use and eliminating as much as possible mechanical impact on fragile scales that might prove damaging.

Another important topic was to elaborate such a method that would be less invasive and harming on the fish, in order to allow consecutive age determinations on the same individual. Even if such methods as otolith, fin ray section or operculum extraction might prove more reliable, they also prove more damaging for the individuals, often involving sacrificing the fish.

### PRINCIPLE OF THE METHOD

The basis of the anatomical methods employed in age determination in fish is represented by observing certain periodical growing marks of bony (rigid) structures of the body, for which an adequate time scale can be established (Jearld A. Jr., 1983). This concept is analogy to the interpretation of annual growth rings in trees,

evidenced in a transversal section throughout the trunk. As well as with trees, fishes have (in temperate areas) a growth pattern observable as contrasting bands visible within certain structures of the organism, such as scales, otoliths, bony fin rays, vertebrae and bones, in general.

Since analysis on any structure other than scales and bony fin rays involve sacrificing the exemplary in order to collect the necessary information, those methods prove to be not suitable in long-term study of growth and ageing for small-size populations, reducing thus the application of the method when working with rare/protected species or species of high economical value.

Nowadays, research efforts are conducted towards developing automatic techniques of age determination with fish, involving sophisticated instruments for image analysis, connected to computers and designed to read growth marks on fish scales. The advantages are represented by increased objectivity, rapid evaluation of measurements and rapid determination of age for large numbers of individuals.

### MATERIALS AND METHODS

We tested the coloration procedures on scales belonging to 3 freshwater fish species: *Carasius gibelio* (Bloch, 1782) *Pseudorasbora parva* (Temminck & Schlegel, 1846) și *Alburnus alburnus* (Linnaeus, 1758), all of them of small size or with small scales. Scales were extracted with a surgical clip, from frozen biological material, following the procedures described in literature (Cărașu, 1952) – the areas above and below the lateral line, beneath the dorsal fin of the fish (Fig. 1). Extracted scales were mechanically cleaned by rubbing against moist filter paper, then degreased by short (1-5 minutes) immersion in ethanol 96%.

Coloration involved immersion of the scales in various aqueous solutions of the following colorants: Mallory Blue (Orange G: Aniline Blue 2: 0,5), Bromcresol Green, Malachite Green Oxalate, Chalcone, Hemalaun (Hematoxiline: potassium alaine alcoholic solution ) and Methyl Orange, followed by washing (5-10 minutes in distilled water) and drying on filter paper.

Subsequently, at least 5 scales from the same individual and employing the same coloration were mounted between two microscope glass slides tightly tied together with surgical thread (in order to firmly press the scales in an as-much-as-possible bi-dimensional form, to reduce optical errors or artifacts).

The samples were examined at microscope (Oc. 10 X; Ob. 2X) IOR and were photographed by means of a Panasonic DMC – L10 LUMIX and Canon Powershot A 510 photo cameras.

The authors' efforts did not focus on annual correlation of the growth rings observed on the scales, but on underlining the areas with more circuli, contrasting with those of less circuli, on an approximate similar surface.

## DISCUSSIONS AND CONCLUSIONS

The best results were obtained utilizing Chalcone and Malachite Green for *Carassius gibelio*, Hematoxilin and Bromcresol Green for *Pseudorasbora parva* and Chalcone for *Alburnus alburnus*.

We observed that relevance of coloration differs in accordance to scale structure, but, nevertheless, the method is reliable and eloquent even in field terrain studies, due to its following advantages:

- no coloration procedure, nor other preparation techniques takes more than 5-7 minutes for each scale, from extraction to observation moment;
- techniques employed are not hi-tech dependent, experiments may be carried out even with low cost devices;
- even at low quality or low resolution photos age determination may still be achieved;
- the method involves minimum damage on the biological material and age determination

experiments may be repeated with the same individuals after a certain period of time;

- when compared to precise and complicated procedures utilized in standard observation, the quality of colored scales is at least similar as relevance in age determination;
- coloration procedure does not involve physical damage on fragile scales.

Despite the obtained results, the authors consider that further research efforts, on more fish species belonging to a wider array of age categories and involving colorations other than proposed ones should be carried out.

Also, specific coloration for different scale structures might prove appropriate.

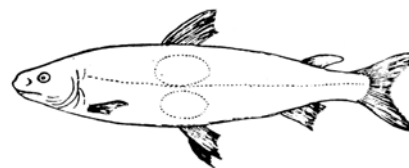


Fig. 1. Areas of interest in scale extraction (Cărușu S., 1952)

## ABSTRACT

The aim of the current paper is to establish a protocol which can be used in determining the age of small-sized fresh water fish, based on direct observation of growth rings (annuli and circuli) within the scale structure.

The method simplifies the currently existing procedures (extraction of a certain number of scales, scouring, polishing, observation) therefore facilitating the age determination process during field activities.

The authors propose a method consisting of extraction, superficial mechanical cleaning and coloration, which considerably shortens the processing time but with good results in microscopy observations.

Another aspect is that small-sized fish have less rigid scales and the risk of mechanical deterioration during polishing procedures is more likely, making them improper for observations.

At the same time simple observations by transparency, after polishing, may be irrelevant.

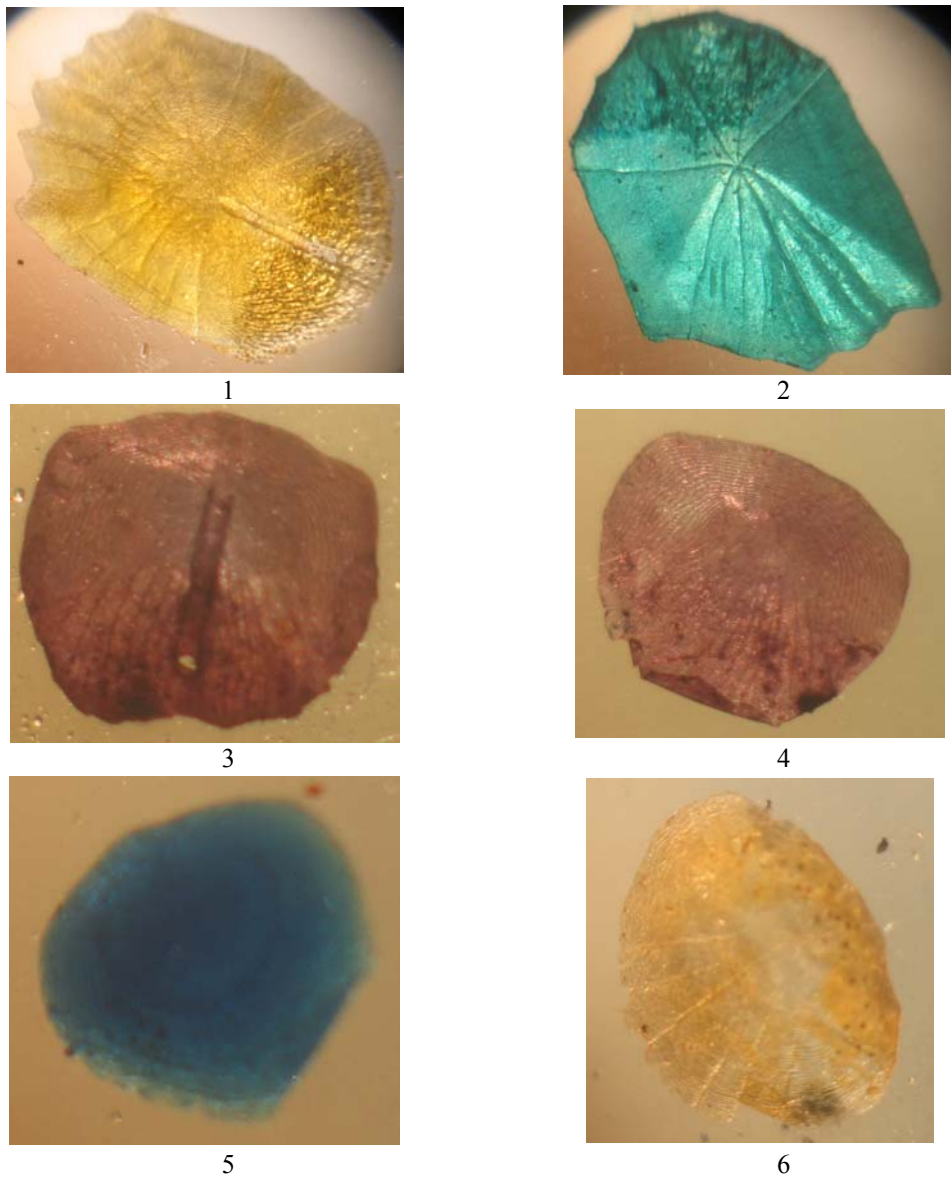


Table 1. Different colorations on three different fish scales: 1. *Carassius gibelio* age 2+, with Chalcone; 2. *Carassius gibelio* age 2+, with Malachite green; 3. & 4. *Pseudorasbora parva* age 3+ with Hematoxylin; 5. *P. parva* age 3+ with Bromocresol green (blurry yet readable); 6. *Alburnus alburnus* age 3+ with Chalcone.

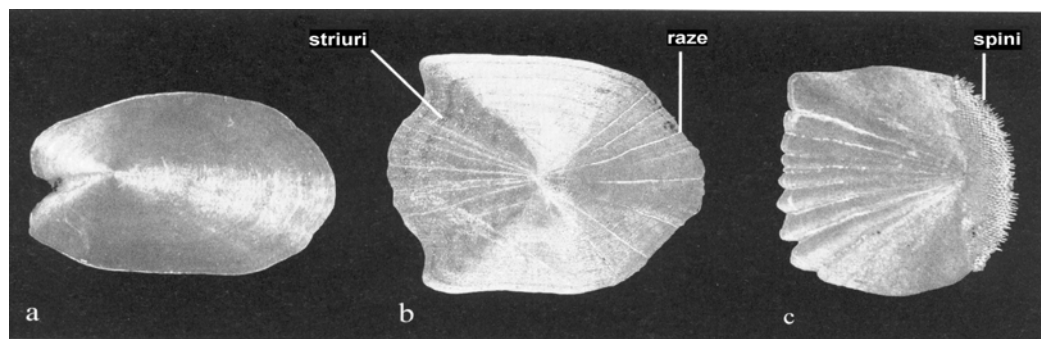


Fig. 2. Aspect of scales in classical (polish&transparency) processing (Kottelat & Freyhof, 2007)

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