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A DESCRIPTION STUDY OF TWO LOCAL FISH HIMRI *CARASOBARBUS LUTEUS* (Heckel, 1843)(CYPRINIFORMES: CYPRINIDAE) AND HISHNI *LIZA ABU* (Heckel, 1843) (MUGILOIDEI : MUGILIDAE) BY BONES STAINING METHOD

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ABSTRACT

Two local fish Himri *Carasobarbus luteus* (Heckel, 1843) and Hishni *Liza abu* (Heckel, 1843) were stained with Alizarin Red and featured some anatomical qualities which cleared the difference of the muscular and skeletal fabric for each fish. Since clear Histologic differences appeared in these two species, it was intended from this study the possibility of adopting a diagnosis between local fish species by staining bones and tissues.

Key words : *Carasobarbus luteus*; *Liza abu*; Cyprinidae; Mugilidae; Baghdad; Iraq.

INTRODUCTION

The method of staining bones is one of the means adopted in the study of tissue and bone, and organs too, through which taxonomic studies can be conducted among species of fish as stated by (Potthoff,1984).as well as differences between taxonomic species of fish known and conventional. Whole fish or some parts of the body such as staining bones and connective tissue could be stain in two colors and clear as pointed out in (Dingerkus and Uhler, 1977).

This protocol was originally modified from Klymkowski and Hanken (1991) for amphibians. Although both cartilage and bone in the same specimen could be stain. It is better to stain bone and cartilage in different specimens of the same developmental stage, (Jonathan Knight, 2009).

Green (1952) stated that alizarin staining method for the preparation of whole skeletons has proved very useful for the study of bones of embryos and small animals. It has certain advantages over methods in which the carcass is macerated and the bones are separated and dried. Among these advantages are: (a) there is no chance of losing the small bones, (b) all bones are retained in their original position, (c) there is no chance of wrongly identifying similar bones, (d) in the finished preparations the bones, after identification, may be disarticulated and examined from all angles, equally as well as in dried preparations, and (e) many animals may be processed together without danger of mixing their bones, a great saving in time and effort.

The main purpose of conducting this study is to identify the possibility of textile and structural differences between two local Iraqi fishes using staining with Alizarin Red.

MATERIALS AND METHODS

Ten local Himri *Carasobarbus luteus* (Heckel, 1843) Fish brought from the local market at Baghdad city at period of collection, Total length of these fish was (12 cm ± 1.5 cm), and

average weight (65 g ± 5 g). also brought ten local Hishni *Liza abu* (Heckel, 1843), The averaged of total length was (14.5 cm ±1 cm) and average of weight (70 g ± 2 g).

The fishes were put in ice water with small ice Cubes, preparing a solution of formalin concentration (10%), to ease the formalin concentration (37%) to the concentration (10%) with adding 3 portion of distilled water to 1 part solution of formalin to get the concentration of formalin (10%), The fish flooded with a solution of formalin diluted Group for more than 48 hours with continuous monitoring, then they washed with water for a full hour to get rid of the remnants of the diluted solution of formalin.

Then potassium hydroxide solution was prepared by dissolving KOH (60 g) KOH crystalls in 1 liter of distilled water, with the preparation of dye alizarin dissolving (0.1 g) in (100 ml) of distilled water. The fish was flooded with a solution of KOH and then added alizarin dye the fish gradually until the arrival of the amount of dye to 50 ml, which is added to a solution of KOH submerging the Fish.

After that the Fish left in staining solution for two days, The scales were removed full of body and quietly and back again to the same KOH solution to stay for Other 5-6 days. a solution of pure Glycerine of more than (70%), was prepared to be placed where the fish is. Immediately after the end of six days in a solution of KOH staining with red alizarin, leaving the fish in Glycerine for 3-4 days. Then bottles were brought to save fish to create an imaging process after Shedding and create the appropriate lighting.

RESULTS AND DISCUSSION

The two studied fishes were identified according to (Khalaf, 1961; Mahdi, 1962 and Forese and Pauly, 2004). The results showed differences in the distribution of the staining on the bones of both fishes. Figure (1) shows the distribution of the staining on the skeleton of *C. luteus*. Figure (2), shows the distribution of the stain on the skeleton of *L.abu*.

Figures (1) and (2) Shows differences textile and distribution of the stain on the skeleton of each species. *L.abu* shoed with pink to red, In addition to the brightness of the color dark purple on the bones of it, while a variation was observed in the distribution of color of bone between the different species, as seen in (1), just like what it was in (Potthoff,1984). The author can distinguish the species of fish from the stain of bone only, as seen from Figure (3). Severity of staining in the bone area near the tail in *C.luteus* which shows the different bone tissue of these fish from *L.abu* as in Figure (4). It was also noticed that the difference in coloration of the bones of the head of each studied fish as it was more pronounced in the first of the second, as in Figure (5).

This shows that the discrepancy in the distribution of the stain between the species probably may be due to the differences of bone tissue between fish species, as show in (Potthoff,1984). While (Klymkowsky and Hanken, 1991) pointed to the raise of the level of clarity when staining, for we can keep the fish in a solution of KOH concentration (1 %), after being placed in formalin and the duration depends on the size of the model, Then the possibility of removing the scales and even the skin gently, to raise the level of staining bones and increase the clarity which was enhanced as stated by (Potthoff,1984) knowing that the second pointed to the possibility stain even fish larvae without resorting to remove scales or remove the skin due to their small size. As for the rest of the small vertebrates such as fish, it was placed in a solution (20 ml acetic acid plus a 80 ml alcohol and 15 mg Alcian blue) then used immediately after immersion to dilute formalin (10%).

Lewis and Witten (2004) referred to the possibility of stain connective tissue and cartilage without muscle tissue after the change in the concentration of formalin and potassium hydroxide and adoption Glycerine purity of up to (100%), and the different periods of immersion fish with the removal of muscle tissue gently to one aspect of the fish intended for study, and came this way also identical with source (Klymkowsky and Hanken, 1991) as in Figure (6), which shows the degree of clarity and form in this way. (Lewis and Witten, 2004) with the length of the fish immersion in staining solution, if the length of the fish is between (1cm - 8cm) dive for a one-day staining solution, but if the length of the fish is between (8cm - 20cm), two days, but if the length of the fish above (20cm) the period should be 4 days of immersion and above depending on the size and the length of the form, in this study the fish dive in staining solution for two days and after removing the scales for 5 more days. Each of the sources (Klymkowsky and Hanken, 1991) and (Jonathan, 2009), point out to the different ways of staining for the previous method, and at the same time for a way to hold staining in this study.

Gavaia and Cancela (2000) noted that the accuracy and clarity of the models are due to differences in the concentrations of the chemicals used, and when additives are used, Then tend to promote the work and raise the level of clarity of form for a taxonomic studies on the fish species studied.

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Fig.1: *Carasobarbus luteus* (Heckel, 1843).

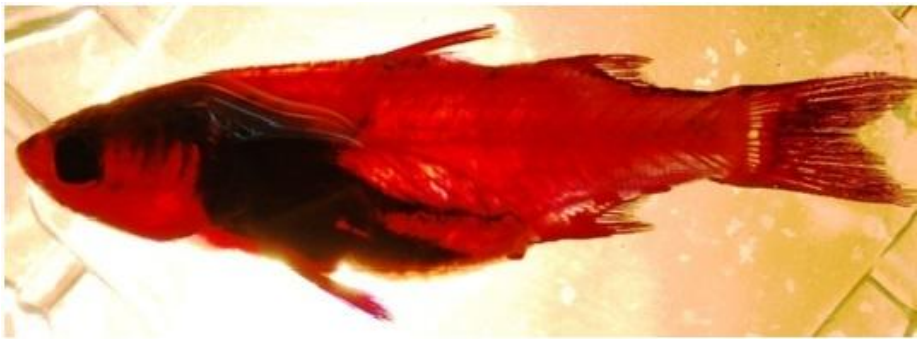

Fig.2: *Liza abu* (Heckel, 1843).

Fig.3: The distribution of the stain and coloration in the tail of *C. luteus* (Heckel, 1843).

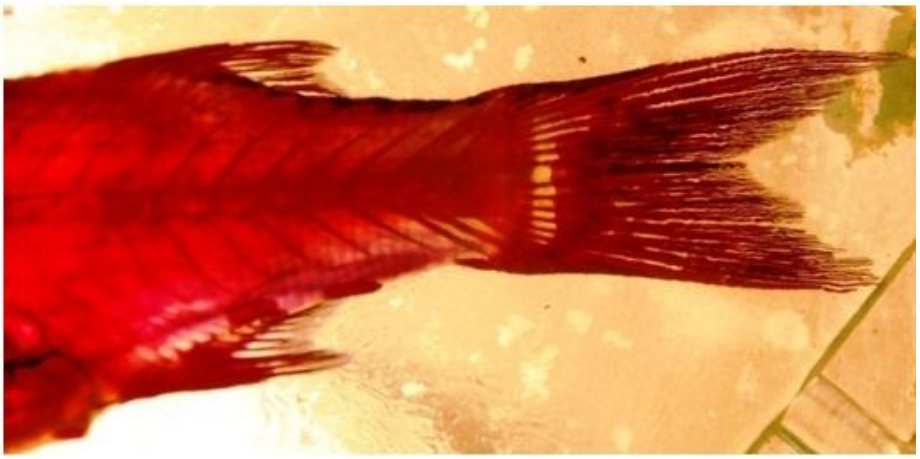

Fig.4: The distribution of the stain and coloration in the tail of *L. abu* (Heckel, 1843).

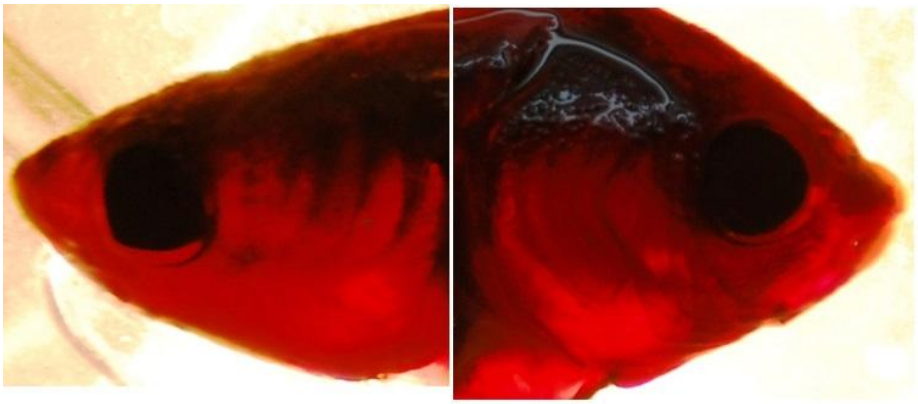

Fig.5: The distribution of the stain and the clarity of head bones of A. *L. abu* and B. *C. luteus*

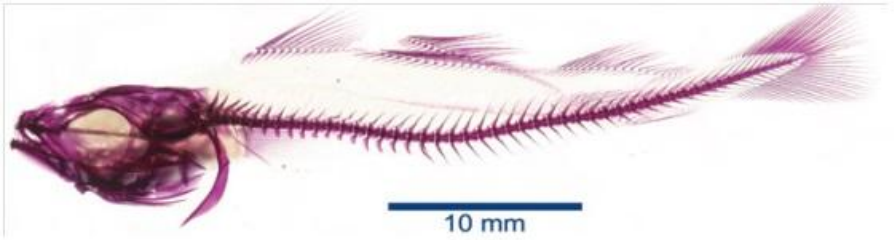

Fig.6: The degree of staining clarity Association of tissues and bones, (Klymkowsky and Hanken, 1991).

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دراسة وصفية لنوعي الاسماك الحمري *CARASOBARBUS LUTEUS* (Heckel, 1843) والخشني : *LIZA ABU* (Heckel, 1843) (MUGILOIDEI : MUGILIDAE) المحلية بطريقة تصبيغ العظام

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الخلاصة

تم تصبيغ كل من الاسماك المحلية الحمري *Carasobarbus luteus* (Heckel, 1843) والخشني *Liza abu* (Heckel, 1843) بصبغة الاليزارين و ظهرت بعض الصفات التشريحية التي يمكن من خلالها دراسة التنوع و الاختلاف بالنسيج العضلي و الهيكل العظمي لكل من هذين النوعين المحليين ، كان الهدف من هذه الدراسة امكانية اعتماد التشخيص بين الانواع السمكية المحلية بطريقة تصبيغ العظام و الانسجة.