Asian J. Exp. Sci., Vol. 23, No. 1, 2009; 199-205

Postmortal Changes in *Clarias gariepinus* (Burchell, 1822)

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Abstract : The present communication deals with the study of the postmortal changes in the skin coloration and muscle myogen of *Clarias gariepinus* (Burchell, 1822).

Key words : *Clarias gariepinus*, SDS - PAGE, Muscle myogen, Chromatophores.

Introduction

The appearance of a newly landed fish is unforgettable. The interplay of subtle shades of beautiful colours make it a joy to behold, and irresistible as an item of food. Just a few hours after death, though it begins to look less obviously attractive and its now 'ordinary' colours are much more familiar to majority of the public. Their is a great deal of intrinsic variation in the colour of fish, depending on where they were caught, and experienced buyers on the market can often tell, where the boats have been and where the fish have been netted. The appearance of fish markedly influence the reddyness with which people buy them, and a merchant neglects this apparently trivial aspect of fish biology at its peril.

Not only this, loss of fish freshness, followed by spoilage is the result of complex microbiological, physiological, chemical and biochemical processes. Initial step in deterioration consist of hydrolytic reactions, catalyzed by endogenous enzymes, which produce nutrients, that permits subsequent bacterial proliferation. Postmortal protein degradation in the muscle cell is probably the result of synergistic action of many enzymes (Ouali *et al.*, 1992; Ogata *et al.*, 1998). Despite of these facts practically nothing is known about the posmortal changes in skin colouration and muscle myogen of *Clarias gariepinus* (Burchell, 1822). Thus, keeping in mind the present study was started to evaluate, the changes takes place in the chromatophores in the skin and in muscle myogen in the white muscles at regular interval of three hours.

Materials and Method

Live fishes, *Clarias gariepinus* for present investigation were obtained from the local fish markets of Meerut and identified with the help of classical works of Mclnerny and Gerard (1958), Misra (1959), Day (1978), Srivastava (1968 and 1980) and Nelson (1994). After the death of fish we remove skin and white muscle for the study of chromatophores and muscle protein respectively. After that they were studied after every three hours interval.

Processing of Skin

All the samples of skin were fixed in 10% neutral Formalin for 24 hours then washed properly in running tap water. After washing, skin were treated in 5% KOH for 1 hour. Permanent mounts of skin were made after staining in Borax Carmine, dehydrating through ascending grades of Alcohol, clearing in Xylene and mounting in Canada Balsam. Microphotographs were also taken with the help of Motic Research Microscope.

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SDS- PAGE Analysis of White Muscle

Fish muscle tissues were homogenized in tissue homogenizer with a motor driver pestle. Homogenates were centrifuged at 1000 rpm for 10 minutes to remove the debris of broken cells etc. Protein concentrations from the sample were determined by the method suggested by Lowry *et al.* (1951). Electrophoresis of proteins in the presence of SDS was carried out as described by Laemmli (1970) with minor modifications. Gels were photographed under transillumination with Canon AE-1 Camera using 100, ASA Kodak Coloured film. Diagrammatic representation of banding pattern was prepared, using computer.

Results

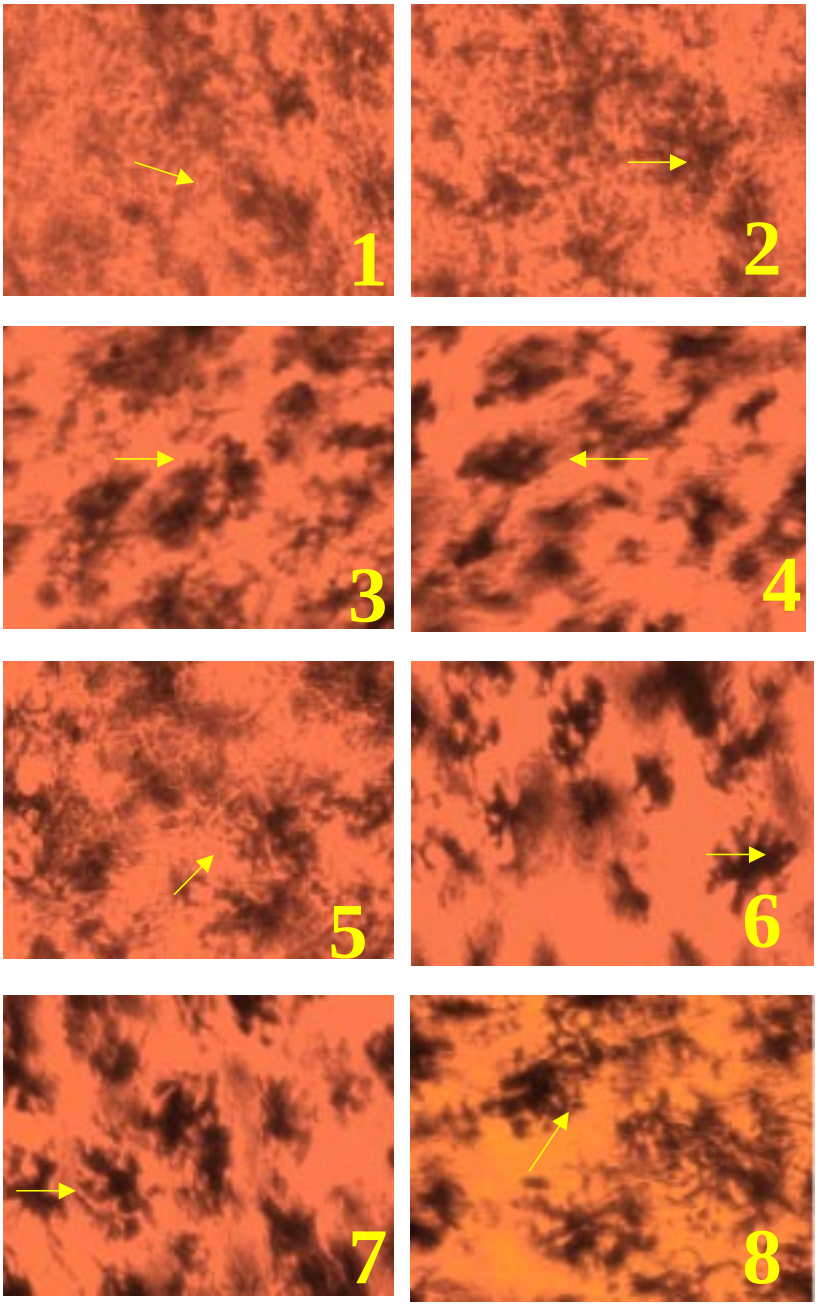
During course of the study of postmortal changes in *Clarias gariepinus* the investigators killed the fish. Skin and muscle samples were taken at the interval of three hours. Salient points of observation were given as follows. **Skin:** With onset of rigor, chromatophores became more bright and visible in comparison to the normal. The size of chromatophores and their morphology became more clear with the increase in time (Plate-I). **Muscle myogen:** Study of muscle myogen is appended in the form of Plate II and Table1. Study of Zymogram reveals that in control their were only 12 bands which increased to 16 after the onset of rigor. However, individual variations were noted in the bands at different intervals of the time. Soon after death at lane number 0 the bands which were present are 1, 2, 3, 6, 7, 9, 14, 15, 16, 17, 18 and 19. However, the bands which were absent in this lane were 4, 5, 8, 10, 11, 12 and 13. After three hours of death, band no. 1 remained absent, together with band no. 4, however, a few new bands appeared when it was compared with the data obtained after three hours of death, the band no. 8.

The remaining scenario remains unchanged except-

Table 1 : Matrix of protein bands of *Clarias gariepinus* (Burchell, 1822)

S.No.	0 hrs	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs
1	1	0	0	0	0	**1**	**1**	**1**
2	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1
4	0	0	**1**	1	1	1	1	1
5	0	**1**	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1
7	1	1	1	1	1	**0**	**0**	**0**
8	0	**1**	0	0	0	0	0	0
9	1	1	1	1	1	1	1	1
10	0	**1**	1	1	1	1	1	1
11	0	**1**	1	1	1	1	**0**	**0**
12	0	0	0	0	0	0	**1**	**1**
13	0	**1**	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1

PLATE- I : Photomicrograph of fish skin of C*larias gariepinus* (Burchell, 1822) x 400.



Photomicrograph 1 – Zero hours.
Photomicrograph 2 – 3 hrs.
Photomicrograph 3 – 6 hrs.
Photomicrograph 4 – 9 hrs.
Photomicrograph 5– 12 hrs.
Photomicrograph 6 – 15 hrs.
Photomicrograph 7 – 18 hrs.
Photomicrograph 8 – 21 hrs.

PLATE- II

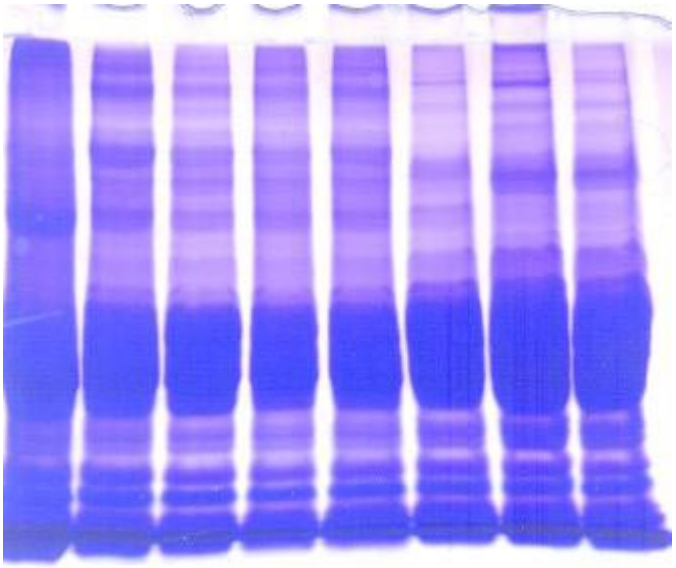


Fig. 1 : Photograph of SDS – PAGE gel of white muscle of *Clarias gariepinus* (Burchell, 1822).

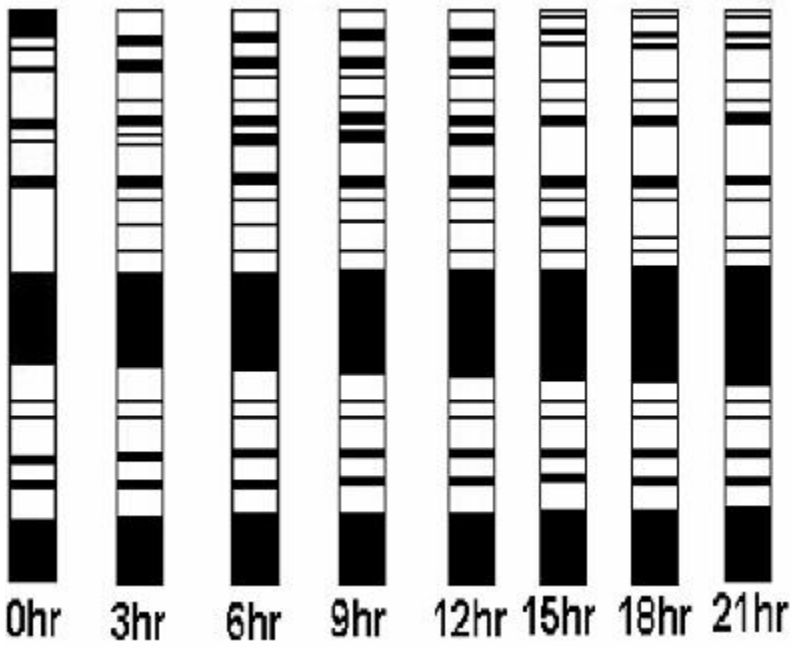


Fig. 2 : Diagrammatic representation of SDS – PAGE gel of white muscle of *Clarias gariepinus* (Burchell, 1822).

1. Band no. 1 appeared again in 15 hrs, 18 hrs and 21 hrs.
2. Band no. 7 disappeared in 15 hrs, 18 hrs and 21 hrs.
3. Band no. 11 disappeared during 18 and 21 hrs.
4. A new, band no. 12 appeared during 18 and 21 hrs.

From the foregoing observations, it is apparently clear as a marker of postmortal changes, band no. 1,7,11 and 12 can be taken into consideration out of these four band no. 1 and 12 appeared for the first time and band no. 7 and 11 which were present in previous observation got disappeared.

Discussion

Over the years numerous reviews concerning the factors responsible for postmortal changes in mammals and birds have been published (Hamn, 1986; Offer and Knight, 1988; Solomon *et al;* 1998; Honikel, 2004; Huff- Lonergan and Lonergen, 2005). But results obtained from studies on other groups of animals may not always apply to fish, since fish are ectothermic organisms with a structural organization quite different from warm blooded animals.

Increase in brightness of melanophores and increase in size of individual chromatophores are due to stopped blood circulation after death. Blood contains variety of humoral factors including adrenaline, which plays important role in contraction and dispersal of chromatophores as also reported by Smith (2004) in *Phoxinus*. Mechanism behind the postmortal changes in fish muscle are not fully understood, but it is assumed that endogenous proteolytic enzymes are important. Skeletal muscle in teleosts run both the sides of body, extending from head to tail and are divided into muscle segments known as myotomes separated from each other by connective tissue strands called myocommatas. Myotomes essentially consist of parallel running muscle fibers that are at each end connected by mycommata by fine collagenous fiber. In addition to connecting the myotomes, the mycommata is also connected to the skeleton and skin.

The SDS-PAGE analysis showed that their were only minor differences in quality of proteins. Sarcoplasmic proteins, which disappeared at lane no. 7 and 11 and appeared at lane no. 1 and 12 are very much detectable. It is difficult for authors to correlate findings with earlier workers as very few references are available in the literature. One thing is very much clear that deterioration of muscle is not due to break down of muscle fibre, but due to proteolytic degradation of minor components probably use to link the structural units together (Olafsdottir *et al.,* 1997).

Mechanisms behind postmortal changes in fish muscle are not fully understood, but it is assumed that endogenous proteolytic enzymes are of importance. These enzymes are chiefly lysosomal cathapsins (Huff- Lonergan and Lonergan, 2005; Geesink *et al.*, 2006; Toyohara and Makinodan, 1989; Geesink *et al.,* 2000; Verrez- Bagnis *et al.,* 2002; Saito *et al.,* 2007; Yamashita and Konagaya, 1990; Aoki and Ueno, 1997; Ho *et al.,* 2000; Ladrat *et. al.,* 2003; Cheret *et al.,* 2007) as well as connective tissue degrading enzymes, like matrix metalloproteniases (Bracho and Haard, 1995; Saito *et al.,* 2000; Delbarre – Ladrat *et al.,* 2006; Olsson *et al.,* 2006), which have been implicated in the postmortem degradation process of fish muscle.

Acknowledgement

We are thankful to the Head, Department of Zoology, C.C. S. University, Meerut, for providing laboratory facilities and valuable suggestions. Authorities of CSIR are also gratefully acknowledged for financial assistance.

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