Comparative Study of Electrophoretic Patterns of Protein sub units in various Tissues of TwoFresh Water Fishes (*Heteropneustesfossilis* and *Labeorohita*)throughGel Electrophoresis

ABSTRACT

The present investigation is determine the comparative study of electrophoretic patterns of protein sub units in two different fishes i.e.*Heteropneustesfossilis* and *Labeorohita*. All the tissues were examined on 7.5% SDS-PAGE.In this investigation we observed gill, liver, intestine , muscle and brain tissues of both fishes. In this Gill and Muscle tissues of two fishes exhibits highest protein bands (10 and 7 respectively).The lowest bands are observed in intestine in both fishes(7,5 respectively).In this investigation we observed homology in protein bands with minor variations.

KEYWORDS: Protein patterns, SDS –PAGE, *Heteropneustesfossilis, Labeorohita,* tissues.

INTRODUCTION

The rising application of pesticides in agriculture, encompassing both commercial and domestic vegetable production for pest management, leads to chemical contamination of aquatic ecosystems. This contamination poses significant health risks to livestock, particularly affecting fish, amphibians, birds, and mammals (K. Sunitha *et al*., 2010). Pesticides are routinely utilized on agricultural products to improve both the quality and quantity of food. However, the unregulated and excessive use of synthetic chemical pesticides results in harmful consequences, including alterations in water odor and taste, lethal impacts on various non-target aquatic organisms, and both direct and indirect effects on users (Kalavathy *et al.,* 2001). Environmental pollutants and their effects on human health(Shilpa Sshetty,et al., 2023).Environmental pollutants have severely impacted fish and diverse wildlife populations. The regular application of industrial chemicals and pesticides adversely affects water bodies and soil quality. Previous studies have documented the detrimental effects of pesticides on mammals, fish, and birds. While the use of these pesticides has led to increased crop yields and a notable decrease in post-harvest losses (Ravichandran *et al.,* 2017), their continuous application has negative implications for human health. Currently, over 1,400 pesticides are utilized globally, including herbicides,

insecticides, acaricides, and fungicides (Turinek*et al.*, 2009). Among synthetic pesticides, organophosphates are the most widely consumed (AI-Sharbati*et al.*, 1998).The majority of agrochemicals utilized in agriculture are resistant to degradation, persisting in aquatic environments for extended durations, which negatively impacts fish and other aquatic organisms (Ramaswamy *et al*., 2007). The physiological and biochemical changes that occur in animals under physiological stress can be linked to alterations in the structure and function of cellular proteins. Proteins play a critical role in cellular metabolism due to the proteinaceous nature of enzymes that facilitate various metabolic pathways. Contemporary agricultural practices necessitate the extensive use of pesticides in pursuit of increased crop yields, leading to the accumulation of these substances in the food chain and resulting in harmful effects on the biomarkers of aquatic life (Yu Zhang *et al*., 2012). One of the most prevalent techniques across various scientific disciplines, including molecular biology, biochemistry, and forensic science, is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which separates proteins in a gel according to the length of their polypeptide chains. Consequently, SDS-PAGE is widely employed to classify proteins based on their electrophoretic mobility. According to (Muhammad 2018), SDS-PAGE analysis serves as a vital tool for toxicological studies involving fish. Additionally, protein electrophoresis has been effectively utilized in numerous research efforts to discern both intra- and inter-specific variations among different species.

MATERIALS AND METHODS

Preparation of SDS-PAGE Gel and Sample Preparation

The samples were subjected to SDS-PAGE utilizing a 6% stacking gel and a 10% separating gel, as outlined by Laemmli (1970). An electrophoretic analysis of protein patterns in the intestinal tissues of Channa punctatus and Labeorohita was conducted on the aforementioned tissues.

Gel Preparation and Casting

The gel plates were assembled following the manufacturer's guidelines, and the volume of the gel mold was calculated. In a conical flask, the acrylamide mixture for the 10% resolving gel was prepared, consisting of 10 ml acrylamide, 7.5 ml resolving gel buffer (pH 8.8), 12.3 ml distilled water, 150 µl freshly prepared ammonium persulfate (10% stock), and 50 µl TEMED (N,N,N,N’

Tetra Methylene diaminine). The components were thoroughly mixed, and the acrylamide mixture was promptly poured into the glass mold up to the designated lower mark. A layer of distilled water or isopropyl alcohol was applied to promote effective polymerization. Following a polymerization period of 30 minutes, the overlay was removed, and the top of the gel was rinsed with distilled water.

Preparation of Stacking Gel

A 6% stacking gel was prepared by combining 2 ml of acrylamide (30% stock), 3 ml of stacking gel buffer (pH 6.8), 4.9 ml of distilled water, 75 µl of ammonium persulfate (10% stock), and 25 µl of TEMED. The components were mixed thoroughly and poured over the resolving gel, with the comb inserted immediately to allow for polymerization.

Sample Loading

Intestinal tissue samples from Channa punctatus and Labeorohita were prepared using the required volume of sample (100 µg protein per lane) combined with an equal volume of sample buffer, which consisted of 7.25 ml distilled water, 1.25 ml stacking gel buffer, 1 ml glycerol, 0.5 ml β-mercaptoethanol, 150 mg SDS, and a pinch of bromophenol blue.

The samples were subjected to a boiling water bath for a duration of two minutes, which resulted in protein denaturation. Subsequently, the samples were placed on ice to maintain this denatured state. The comb was then carefully extracted from the mold, and the wells were rinsed with distilled water. The gel was positioned onto the electrophoretic apparatus. Electrophoretic buffer, composed of 3 grams of Tris, 14.4 grams of Glycine, and 1 gram of SDS dissolved in 1000 milliliters of distilled water at pH 8.3, was introduced into both the top and bottom reservoirs of the apparatus. The samples, along with marker proteins, were loaded into the designated lanes or wells.

The apparatus was connected to a power supply unit, applying a voltage of 8 volts per centimeter for the gel (70 volts) and 15 volts per centimeter for the resolving gel (150-200 volts). Care was taken to ensure that there were no air bubbles present while adding the electrode buffer to the tanks, as these bubbles could impede electrophoretic mobility. The gel was allowed to run until

the Bromophenol blue dye reached the bottom of the resolving gel. Once this was achieved, the power supply was turned off, and the gel was carefully removed from the sandwiched plates of the apparatus and placed on a paper towel. The plates were detached using a spatula, and their orientation was marked. The temperature during electrophoresis was maintained at a constant 25°C in an air-conditioned environment.

For staining and destaining, the gel was immersed in a staining solution consisting of 200 mg of Coomassie Brilliant Blue R, 250 ml of methanol, 50 ml of acetic acid, and 63 ml of distilled water, with the total volume adjusted to 300 ml. This immersion was repeated every half hour, followed by two to three washes. The gel was subsequently stored in a 7% acetic acid solution. Upon visualization under illumination, the protein zones appeared as dark blue bands after a destaining period of 24 to 48 hours. The results were documented by assessing the relative electrophoretic mobility of the protein zones for each sample, and the procedure was repeated for samples that did not exhibit distinct zones. The protein profiles obtained from the prepared cell lysates were manually analyzed and compared with the various protein bands present in the standard, control, and treated samples.

RESULTS

Protein bands in *Heteropneustesfossilis*

Gill

The gill showed 10 protein bands with Rm values 0.23,0.41,0.48,0.55, 0.61, 0.65, 0.70, 0.73 0.76 and 0.86. In these three bands are in Zone-A(100-70KDa), four bands are inZone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Liver

The liver showed 09 protein bands with Rm values 0.01,0.05,0.23, 0.41, 0.48, 0.53,0.75, 0.86, 1.0; In this three bandsare in Zone-A(100-70KDa),four bands are in Zone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Intestine

The intestine showed 07 protein bands with Rm values 0.25,0.40, 0.50, 0.66, 0.75, 0.88 and 0.98. In this two bands are inZone-A(100-70KDa), three bands are in Zone-B(55-35KDa), remaining two bands are in Zone-C(34-5KDa).

Muscle

The muscle showed 10 protein bands with Rm values 0.06,0.26, 0.38, 0.46, 0.55, 0.70, 0.76, 0.80, 0.86 and 0.96. In thisthree bands are in Zone-A(100-70KDa), four bands are inZone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Brain

The brain showed 08 protein bands with Rm values 0.11,0.23, 0.36, 0.50, 0.63, 0.73, 0.83 and 0.86. In this two bandsare in Zone-A(100-70KDa),four bands are in Zone-B(55)

Table. I R_m values of all tissue protein patterns in*Heteropneustesfossilis*

Standard ProteinMarker	G	L	I	M	B
	0.01	**0.01**	**0.01**		**0.01**
				0.04	
		0.05		**0.05**	**0.05**
0.23	**0.23**	**0.23**	**0.16**	**0.16**	
0.38				**0.21**	**0.20**
	0.41	**0.41**			**0.23**
0.48	**0.48**	**0.48**			**0.36**
	0.53	**0.53**			
		0.66			
	0.58	**0.58**			
	0.61	**0.86**	**0.83**		**0.53**
0.65					**0.58**
0.70					**0.63**

		1.0	**1.0**	**0.66**	
0.83	**0.83**	0.83			
				0.76	
				0.78	
				0.80	
				0.86	
				0.91	

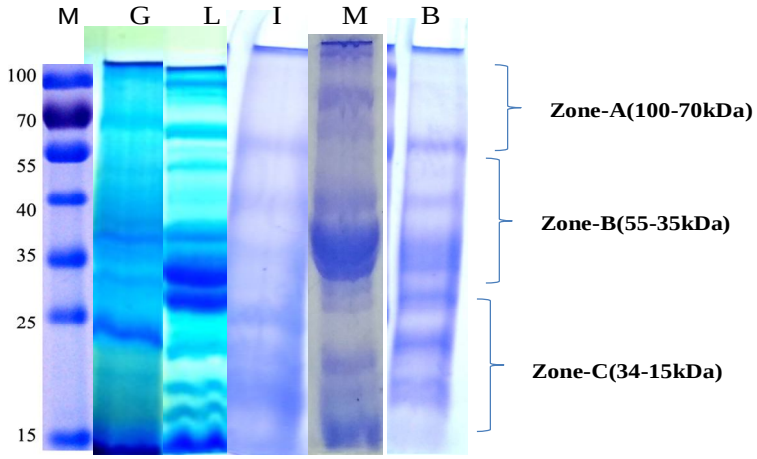


Fig.1. Comparative study of electrophoretic patterns of Proteins in different tissues of *H.fossilis*.

Protein bands in *Labeorohitha*

Gill

The gill showed 7protein bands with Rm values 0.06,0.023,0.38,0.41,0.55,0.65 and 0.70. In these three bands are in Zone-A(100-70KDa), three bands are in Zone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Liver

The liver showed 06 protein bands with Rm values 0.05,0.23, 0.41, 0.53.0.65,and 0.70, In this two bands are in Zone-A(100-70KDa),three bands are in Zone-B(55- 35KDa), remaining three bands are in Zone-C(34-15KDa).

Intestine

The intestine showed 05 protein bands with Rm values 0.01, 0.05,,0.41, 0.65 and 1.0 . In this two bands are in Zone-A(100-70KDa), two bands are in Zone-B(55- 35KDa), remaining two bands are in Zone-C(34- 5KDa).

Muscle

The muscle showed 7 protein bands with Rm values 0.01,0.05,0.410.53,0.58 ,0.65,0.70 In this three bands are in Zone-A(100-70KDa), three bands are in Zone-B(55-35KDa), remaining three bands are in Zone- C(34-15KDa).

Brain

The brain showed 06 protein bands with Rm values 0.01,. 0.06, 0.23, 0.36, 0.50, and 0.70 . In this two bands are in Zone-A(100-70KDa),four bands are in Zone-B(55)

Table. 2 R_m values of all tissue protein patterns in*Labeorohita*

Standard ProteinMarker	G	L	I	M	B
	0.06		**0.01**	**0.01**	**0.01**
		0.05		**0.05**	**0.06**
0.23	**0.23**	**0.23**	**0.05**		**0.23**
0.38	**0.38**				**0.36**
		0.41	**0.41**	**0.41**	
0.48	**0.48**				
	0.55	**0.53**		**0.53**	
				0.58	**0.50**
	0.65		**0.65**	**0.65**	

0.65		**0.65**			
0.70	**0.70**	**0.70**		**0.70**	**0.70**
			1.0		

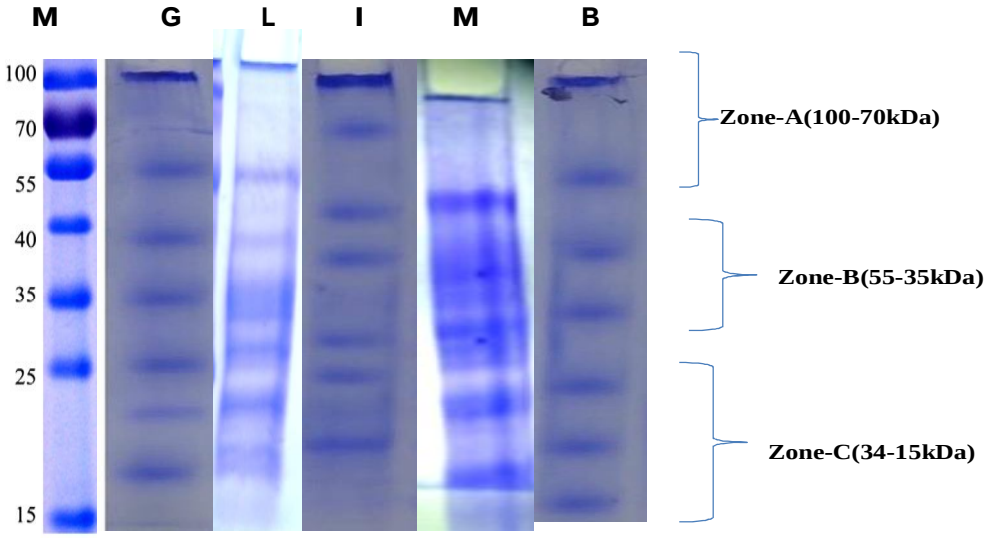
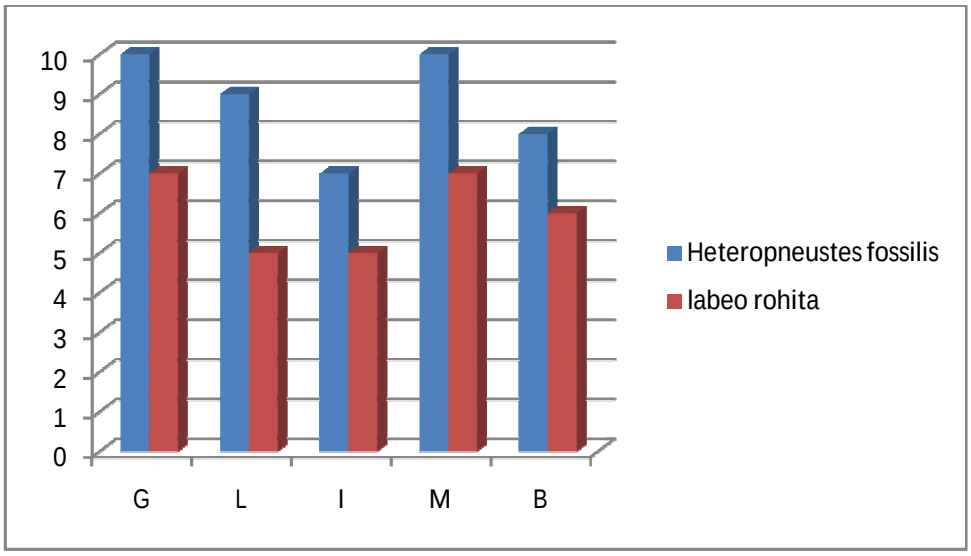


Fig.2. Comparative Study of Electrophoretic Patterns of Proteins in various Tissues of Fresh Water *fish Labeorohita*



Graph1. Graphical representation of total protein bands in different tissues in fresh water fishes*Heteropneustesfossilis*and *Labeorohita*

DISCUSSION

The electrophoretic analysis of both gill and muscle tissues from *Heteropneustesfossilis* revealed similarities in protein banding patterns, albeit with minor discrepancies. It is important to note that electrophoresis does not confirm the identity of two proteins; rather, it highlights their differences. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a widely utilized method in the fields of biochemistry, genetics, and molecular biology for the separation of proteins based on their electrophoretic mobility. Historically, the identification of fish species relied primarily on the examination of external morphological traits. In contemporary research, the electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins, and various enzymes has increasingly been employed by some scientists as a supplementary tool for fish species A comparative analysis of total muscle protein content among *Anabas testudineus, Labeogonius, Labeorohita, and Heteropneustesfossilis,* along with an examination of their electrophoretic banding patterns using SDS-PAGE, was conducted by Dipika Doloi*et al.* (2020). Acute Toxicity Studies On The Fresh Water Fish *Clarias Batrachus*(Helan Chandra 2015.)Exposed To Pesticide Rogorin J Histological and Protein Profile Alterations ofLiver of Freshwater Fish *Heteropneustesfossilis* (Bloch) on Exposure to Fungicide, Fytran),(Deepasree M .I , M. S.

Rajendran).Additionally, the sub-lethal effects of methyl parathion on the protein content of various tissues in *Channa gachua* were investigated (Jain Shampa, 2014). The impact of methyl parathion on the electrophoretic patterns of proteins in the brain, intestine, and liver tissues of the freshwater catfish Heteropneustesfossilis was explored by Bheem Rao *et al*. (2022), followed by a similar study in 2023. Furthermore, a comparative study of the electrophoretic patterns of proteins across five tissues of the freshwater catfish Channa punctatus was conducted by Bheem Rao *et a*l in 2024.Lethal And Sublethal Effects Of Profenofos And Carbosulfan On ProteinPattern Of Indian Major Carp, Labeo Rohita (Hamilton)(Nagaraju*et al*., 2016).

CONCLUSION

In the present investigation we observed more number of protein sub unites in gill and muscle tissues of both fishes.The observed changes in protein bands across different tissues of treated fish, along with the specific protein differences among individuals within a species group, may help clarify taxonomic issues concerning disputed species. In the context of electrophoretic techniques, closely related species exhibit numerous shared electrophoretic alleles, while also displaying fixed differences in certain alleles. When two distinct species possess an identical number of electrophoretic fractions, a more detailed analysis of the relative mobility of one or more bands may uncover clearly defined species-specific distinctions.

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