Contents lists available at NCBI

The American Journal of Science and Medical Research

Journal homepage: https://ajsmrjournal.com/

Research Article

Comparative Study of Tissue-Specific Esterase Isoenzyme Patterns in Two Freshwater Teleosts: *Catla catla* and *Tilapia mossambica* of different orders

Vailla Rajaiah[1] and Vynala Vimala[2]

[1]*Department of Zoology, Kakatiya Government College, Hanamkonda, India.*
[2]*Department of Zoology, TGSWRDC (W), Warangal West*

**Corresponding author:*
E-mail: rajaiahvailla@gmail.com

https://dx.doi.org/10.5281/zenodo.21299658

Received: 29 April 2026
Revised: 22 June 2026
Published: 29 June 2026

ISSN: 2377-6196© 2025 The Authors.
Published by AIRA

Keywords: *Catla catla, Tilapia mossambica, Esterase Isoenzymes, Zymogram, Rm Value, Biomarkers, Tissue Specificity.*

ABSTRACT

Esterases are a diverse group of hydrolytic enzymes involved in vital physiological processes such as detoxification, neurotransmission, and the metabolism of endogenous and xenobiotic compounds, and they are widely recognized as sensitive biochemical markers in ecotoxicological and comparative studies. The present investigation aimed to analyze the tissue-specific distribution and electrophoretic mobility (Rm values) of esterase isoenzymes in two freshwater teleost fishes belonging to different taxonomic orders, *Catla catla* (Cypriniformes) and *Tilapia mossambica* (Perciformes). Zymogram analysis using native polyacrylamide gel electrophoresis was carried out on six tissues—gill, liver, intestine, muscle, brain, and eye—and the detected isoenzymes were classified into Carboxylesterases (CE), Esterase-resistant to inhibtors (ER), Cholinesterases (ChE), and Choline-esterase like enzmes (CHsp), types based on their sensitivity to specific inhibitors such as paraoxon and eserine. The results revealed clear interspecific and tissue-specific differences in esterase profiles, with *C. catla* exhibiting a more complex pattern comprising six isoenzyme zones (Rm values: 0.96, 0.86, 0.76, 0.56, 0.45, and 0.33), whereas *T. mossambica* showed a simpler pattern with three zones (Rm values: 0.96, 0.75, and 0.66). In *C. catla*, the Rm 0.56 zone was consistently observed across all tissues but displayed functional variation, acting as CE in the liver and ER in the gill, indicating tissue-dependent biochemical roles; similarly, in *T. mossambica*, the Rm 0.75 zone was ubiquitous, functioning as CE in metabolically active tissues such as liver and brain and as CHsp in muscle and intestine. Organ-specific expression was also evident, with unique high-mobility zones (Rm 0.96 and 0.86) in the intestine of *C. catla*, while in *T. mossambica*, the Rm 0.96 band appeared as a common marker in gill, liver, and intestine. Notably, isoenzymes sharing similar electrophoretic mobility often differed in their inhibitor sensitivity across tissues, suggesting the presence of distinct tissue-specific isoforms. Overall, the findings demonstrate that esterase isoenzyme patterns are both species-specific and tissue-dependent, reflecting underlying physiological adaptations, and underscore their potential application as reliable biomarkers for monitoring environmental pollution and assessing the impact of organophosphate and carbamate pesticides in aquatic ecosystems.

1. Introduction

Electrophoresis has emerged as an indispensable tool for analyzing protein and enzyme diversity across different tissues of the same species and for comparing homologous tissues among related organisms (Smith, 1976; Utter, 1985). The technique separates molecules based on their electrical charge and molecular size, with polyacrylamide gel electrophoresis offering particular advantages due to its adjustable porosity that enables sharp separation of protein mixtures (Lakshmipathi, 1994). Esterases (E.C. 3.1.1.x) constitute a complex group of hydrolytic enzymes that catalyze the cleavage of ester bonds into corresponding acids and alcohols. These enzymes participate in numerous physiological processes including nervous impulse transmission, reproduction,

development, detoxification, and xenobiotic tolerance (Rao et al., 1998; Hasan et al., 2006). Their ubiquitous distribution and extensive polymorphism make them excellent biomarkers for environmental pollution monitoring and valuable gene markers across diverse organisms (Bornscheuer, 2002).The heterogeneity and multiplicity of esterases surpass that of most other enzyme systems, with single tissues sometimes exhibiting thirty or more heteromorphs (Masters and Holmes, 1975). A notable characteristic is that homologous tissues from different species, even closely related ones, display wide divergence in both the extent of heterogeneity and the properties of multiple enzyme forms (Masters and Holmes, 1974). This diversity has proven valuable for taxonomic studies, species identification, and understanding evolutionary relationships.Aldridge (1953) first distinguished esterases into 'A' and 'B' types using organophosphate inhibitors, with 'B' esterases being sensitive and 'A' esterases capable of hydrolyzing these compounds. Subsequent work by Bergmann et al. (1957) identified 'C' esterases resistant to organophosphates. Augustinson (1961) and Holmes and Masters (1967) developed comprehensive classification systems based on inhibitor sensitivity, categorizing esterases into carboxylesterases (CE, E.C. 3.1.1.1), arylesterases (ArE, E.C. 3.1.1.2), acetylesterases (E.C. 3.1.1.6), and cholinesterases (including acetylcholinesterases E.C. 3.1.1.7 and pseudocholinesterases E.C. 3.1.1.8).

Later studies, particularly in fishes, revealed esterases exhibiting inhibition patterns that could not be accommodated within this framework, leading to the recognition of additional classes including ER esterases (resistant to all inhibitors), Esdp esterases (sensitive to organophosphates and pCMB), Ese esterases (sensitive to eserine alone), and CHsp esterases (sensitive to all three inhibitors) (Hart and Cook, 1976; Haritos and Salamastrakis, 1982; Lakshmipathi and Reddy, 1989).Early investigations of fish esterases focused primarily on serum polymorphisms. Nyman (1965) documented inter- and intraspecific variations in serum esterases of freshwater roach (Leuciscus rutilus). Sprague (1967) identified polymorphic serum esterases in three Pacific tuna species, attributing variations to multiple alleles within populations. Koehn and Johnson (1967) and Koehn (1969, 1970) demonstrated latitudinal variations in allelic distribution of serum esterases in catostomid fishes, correlating activity preferences of different alleles with habitat temperatures and implicating natural selection in maintaining polymorphisms.

Tissue esterase studies gained momentum in the late 1960s. Ecobichon and Israel (1967) characterized esterases in electric tissue of Electrophorus, identifying acetylcholinesterases, pseudocholinesterases, and carboxylesterases. Holmes et al. (1968) conducted comparative studies across vertebrate tissues, including catfish, establishing the foundation for tissue-specific esterase analysis. Hart and Cook (1976) provided detailed characterization of tissue esterases in two Brachydanio species, introducing new esterase classes and demonstrating both tissue and species specificity.In the Indian context, despite the rich ichthyofaunal diversity, electrophoretic studies on fish esterases remain limited. Rajaiah (1996) comprehensively studied tissue esterase patterns in thirteen freshwater fishes from Perciformes, Siluriformes, and Atheriniformes orders. Chatterjee (1989) documented species-specific esterase variations in five Channa species. However, comparative studies between species from different orders are scarce. Catla catla (Hamilton, 1822), belonging to Cypriniformes order (family Cyprinidae), represents one of the most important Indian major carps, contributing significantly to freshwater aquaculture. Tilapia mossambica (Peters, 1852), belonging to Perciformes order (family Cichlidae), has become globally distributed and is recognized as a problematic invasive species in new warm water habitats. These species differ markedly in their evolutionary history, ecological requirements, and physiological adaptations, making them ideal candidates for comparative biochemical studies.

The present investigation aims to: (1) characterize tissue-specific esterase patterns in six tissues (gill, liver, intestine, muscle, brain, and eye) of C. catla and T. mossambica; (2) classify individual esterase zones based on inhibitor sensitivity; (3) compare the distribution of different esterase classes between homologous tissues; and (4) evaluate the potential of esterase patterns as biochemical markers for species identification and taxonomic studies.

1. Materials and Methods

1.1 *Collection and Maintenance of Experimental Animals*

Live specimens of Catla catla (body weight: 500-800g) and Tilapia mossambica (body weight: 50-100g) were collected from freshwater ponds located within a 60 km radius of Kakatiya University Campus, Warangal (18°00'N, 79°35'E), Telangana, India. Fish were captured using cast nets with the assistance of local fishermen and immediately transported to the laboratory in large plastic containers filled with aerated pond water.

Fish were acclimatized to laboratory conditions for one week in glass aquaria (100L for C. catla, 50L for T. mossambica) maintained at ambient temperature (26-28°C) with natural photoperiod. They were fed ad libitum with natural plankton collected from their native habitats. Water quality parameters (pH 7.2-7.6, dissolved oxygen 5.5-6.5 mg/L) were monitored regularly, and 50% water exchange was performed every 48 hours.

1.2 *Tissue Collection and Homogenate Preparation*

Fish were immobilized by a sharp blow to the head, and six tissues—gill, liver, intestine, muscle (epaxial), brain, and eye (retina)—were rapidly dissected on an ice-cold platform. Tissues from 3-6 individuals (depending on size) were pooled to minimize individual variation and obtain representative patterns for each species.

Table 1. Tissue homogenate concentrations

Tissue	Concentration (w/v)
Gill	10%
Liver	10%
Intestine	10%
Muscle	10%
Brain	10%
Eye (retina)	10%

Tissues were homogenized in chilled 0.01M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl using a motor-driven glass-Teflon homogenizer. Homogenates were transferred to ice-jacketed centrifuge tubes and centrifuged at 2,000 rpm for 10 minutes in a clinical centrifuge at room temperature. The supernatants were collected and mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as tracking dye. Aliquots (0.1 mL) of this mixture were used for electrophoretic separation.

1.3 *Polyacrylamide Gel Electrophoresis*

Vertical slab gel electrophoresis was performed on 7.5% polyacrylamide gels (2mm thickness) using a custom-built apparatus with 10-sample capacity.

Table 2. Composition of gel solutions

Solution	Components	Concentration
A	Acrylamide + Bisacrylamide	15 g acrylamide + 540 mg Bis in 50 mL distilled water
B	Gel buffer	28 g glycine + 6 mg Tris in 1000 mL water (pH 8.3)
C	TEMED	0.28% in distilled water
D	Ammonium persulfate	0.14% in distilled water (freshly prepared)

Stock solutions were mixed in the ratio A:B:C:D = 2:1:1:4 and degassed using a vacuum pump before being poured into glass plate assemblies (14 cm × 14 cm) separated by 2 mm thick spacers. Gel polymerization was allowed for 45 minutes at room temperature.

Samples (0.1 mL) were loaded directly into pre-formed slots (5 mm²) on the separating gel. The remaining space was filled with electrode buffer (gel buffer diluted 1:9 with distilled water). The apparatus was connected to a regulated power supply and placed in a refrigerator (10°C) to maintain constant temperature during electrophoresis.

Electrophoresis was conducted at constant current: 20 mA for the first 15 minutes, followed by 40 mA until the tracking dye migrated 12 cm from the origin. This distance was maintained constant across all experiments to ensure reproducible relative mobility (Rm) calculations.

1.4 *Staining of Esterases*

Gels were stained following the method of Holmes and Masters (1967) as described by Lakshmipathi and Reddy (1989).

Table 3 Staining mixture composition

Component	Stock Solution	Volume Used
Substrate	1% α-naphthyl acetate in 50% acetone	1.5 mL
Buffer	0.5M Tris-HCl (pH 7.4), diluted 1:4	18.5 mL
Dye	0.25% Fast Blue RR salt in water	10.0 mL
Total		30.0 mL

The stain was poured directly onto gels and incubated at room temperature in darkness for 20 minutes. Esterase bands appeared as dark brown zones within 10 minutes. After staining, gels were rinsed in distilled water and fixed in 7.5% aqueous acetic acid.

Relative mobility (Rm) of each zone was calculated as:
Rm = (Distance migrated by esterase zone from origin) / (Distance migrated by tracking dye from origin) × 100

2.5 Inhibitor Studies

Three inhibitors were used to classify esterase zones:
Paraoxon (diethyl-4-nitrophenyl phosphate): 2×10^{-5} M (organophosphate)
Physostigmine (eserine): 10^{-4} M (carbamate)
pCMB (parachloromercuribenzoate): 10^{-4} M (thiol-active compound)

Before staining, gels were pre-incubated for 30 minutes in diluted Tris-HCl buffer (pH 7.4) containing the appropriate inhibitor concentration. Staining was then performed in the presence of the same inhibitor concentration to prevent reversibility of inhibition. Complete disappearance of a band was considered as total inhibition by that particular inhibitor.

2.6 Classification of Esterases

Esterases were classified based on their sensitivity patterns to the three inhibitors, following the schemes of Holmes and Masters (1967), Hart and Cook (1976), Haritos and Salamastrakis (1982), and Lakshmipathi and Reddy (1989).

Table 4 Classification scheme based on inhibitor sensitivity

Esterase Class	pCMB	Eserine	Paraoxon	Description
CE (Carboxylesterases)	+	+	–	Sensitive to paraoxon only
ArE (Arylesterases)	–	+	+	Sensitive to pCMB only
ChE (Cholinesterases)	+	–	–	Sensitive to paraoxon and eserine
ER (Resistant esterases)	+	+	+	Resistant to all inhibitors
Esdp	–	+	–	Sensitive to paraoxon and pCMB
Ese	+	–	+	Sensitive to eserine alone
CHsp (Cholinesterase-like)	–	–	–	Sensitive to all inhibitors

Key: + = resistant (not inhibited); – = sensitive (inhibited)

2.7 *Activity Scoring*

Esterase activity was scored semi-quantitatively based on band intensity:

+++ : High activity (intense staining)
++ : Moderate activity (clear but not intense)
+ : Low activity (faint but visible)
± : Very low activity (barely visible)
2.8 Reproducibility and Data Presentation

All experiments were repeated at least three times with different batches of fish to ensure reproducibility. The patterns presented represent an average of patterns obtained from 10-15 individuals of each species collected from the same pond to minimize inter-population variation. Individual variations

Table 6. Inhibitor sensitivity and classification of esterases zones in *Catla catla*

Tissue	Rm Value	Activity	pCMB	Eserine	Paraoxon	Classification
Gill	0.56	+	+	+	+	ER
	0.45	++	+	+	+	ER
	0.33	+	–	–	–	CHsp
Liver	0.76	+	+	+	+	ER
	0.56	+++	+	+	–	CE
	0.45	+++	+	+	+	ER
	0.33	++	+	–	–	ChE
Intestine	0.96	+	+	+	+	ER
	0.86	+	+	+	–	CE
	0.56	++	+	–	–	ChE
	0.33	++	+	–	–	ChE
Muscle	0.56	+	–	–	–	CHsp
	0.45	++	–	–	–	CHsp
Brain	0.76	++	–	–	–	CHsp
	0.56	+++	–	+	–	ArE
	0.45	++	–	–	–	CHsp
Eye	0.56	++	+	+	–	CE
	0.33	++	+	+	–	CE

Key: + = resistant; – = sensitive; +++ = high activity; ++ = moderate activity; + = low activity

observed during preliminary studies were minimal and did not obscure species-characteristic patterns.

2. RESULTS

2.1 *Esterase Patterns in Catla catla*

Catla catla exhibited six distinct esterase zones with Rm values of 0.96, 0.86, 0.76, 0.56, 0.45, and 0.33. Each tissue displayed a characteristic pattern with tissue-specific distribution of different esterase classes.

3.2 Tissue-Specific Distribution in Catla catla

Table 7: Tissue-specific distribution of esterases in Catla catla

Tissue	Rm 0.96	Rm 0.86	Rm 0.76	Rm 0.56	Rm 0.45	Rm 0.33
Gill				+ (ER)	++ (ER)	+ (CHsp)
Liver			+ (ER)	+++ (CE)	+++ (ER)	++ (ChE)
Intestine	+ (ER)	+ (CE)		++ (ChE)		++ (ChE)
Muscle				+ (CHsp)	++ (CHsp)	
Brain			++ (CHsp)	+++ (ArE)	++ (CHsp)	
Eye				++ (CE)		++ (CE)

Observations

The zone with Rm 0.56 was present in all six tissues but exhibited different inhibitor sensitivity patterns across tissues: CE in liver and eye, CHsp in muscle, ER in gill, ArE in brain, and ChE in intestine.

The zone with Rm 0.45 appeared in gill, liver, muscle, and brain with ER (gill, liver) and CHsp (muscle, brain) characteristics. The zone with Rm 0.33 was present in gill, liver, intestine, and eye, classified as CHsp (gill), ChE (liver, intestine), and CE (eye). ER esterases were most widely distributed, followed by CHsp and CE esterases.

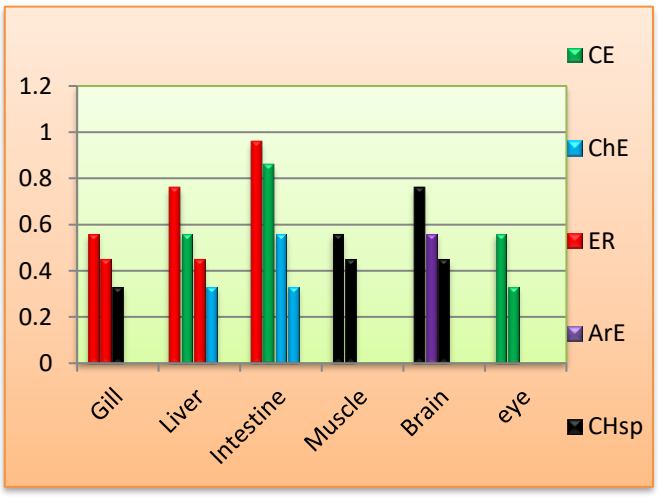

Fig.1 Tissue specific distribution of esterases in Catla catla

3.4 Esterase Patterns in Tilapia mossambica

Tilapia mossambica exhibited three esterase zones with Rm values of 0.96, 0.75, and 0.66. The patterns were simpler compared to *C. catla* but showed distinct tissue-specific variations.

3.4.1 Inhibitor Sensitivity of Individual Zones

Table 8 Inhibitor sensitivity and classification of esterase zones in Tilapia mossambica

Tissue	Rm Value	Activity	pCMB	Eserine	Paraoxon	Classification
Gill	0.96	+	+	+	–	CE
	0.75	++	+	+	+	ER
Liver	0.96	++	+	+	–	CE
	0.75	+++	+	+	–	CE
	0.66	++	+	+	+	ER
Intestine	0.96	+	+	+	–	CE
	0.75	+++	–	–	–	CHsp
	0.66	++	+	+	+	ER
Muscle	0.75	+	–	–	–	CHsp
	0.66	+	+	–	–	ChE
Brain	0.75	+++	+	+	–	CE
	0.66	++	+	–	–	ChE
Eye	0.75	++	+	–	–	ChE
	0.66	+	+	–	–	ChE

3.4.2 Tissue-Specific Distribution in Tilapia mossambica

Table 9. Tissue-specific distribution of esterases in Tilapia mossambica

Tissue	Rm 0.96	Rm 0.75	Rm 0.66
Gill	+ (CE)	+ (ER)	
Liver	++ (CE)	+++ (CE)	++ (ER)
Intestine	+ (CE)	+++ (CHsp)	++ (ER)
Muscle		+ (CHsp)	+ (ChE)
Brain		+++ (CE)	++ (ChE)
Eye		++ (ChE)	+ (ChE)

Observations

The zone with Rm 0.96 was present in gill, liver, and intestine, consistently classified as CE in all three tissues. The zone with Rm 0.75 was present in all six tissues but showed variable classification: CE in liver and brain, CHsp in muscle and intestine, ER in gill, and ChE in eye. The zone with Rm 0.66 was present in five tissues (all except gill), appearing as ER in liver and intestine, ChE in muscle, brain, and eye. CE esterases were predominant in liver and brain, ChE esterases in eye and excitable tissues, and ER esterases in tissues exposed to external environment (gill, intestine).

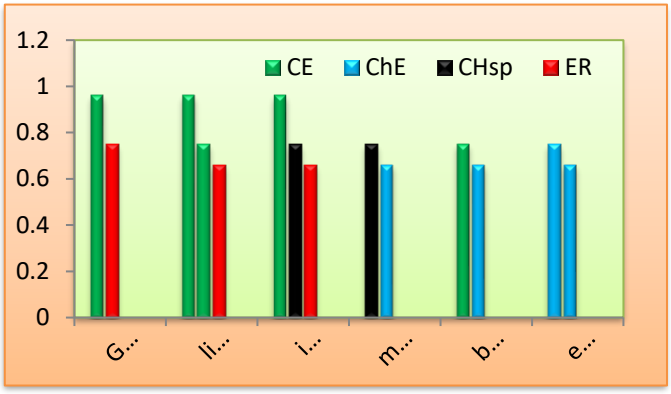

Figure-2. Tissue specific distribution of esterases in *Tilapia mossambica*

3.5 Comparative Analysis

3.5.1 Number of Esterase Zones

Catla catla exhibited twice the number of esterase zones compared to T. mossambica, indicating greater esterase multiplicity in the cyprinid species.

3.5.2 Tissue-wise Distribution of Esterase Zones

Table 10 Number of esterase zones in different tissues

Tissue	*Catla catla*	*Tilapia mossambica*
Gill	3	2
Liver	4	3
Intestine	4	3
Muscle	2	2
Brain	3	2
Eye	2	2
Total unique zones	6	3

Liver and intestine showed the highest number of zones in both species, consistent with their metabolic and detoxification functions.

3.5.3 Comparative Distribution of Esterase Classes

Table 11 Distribution of esterase classes across tissues

Tissue	Species	CE	ChE	CHsp	ER	ArE	Total
Gill	*C. catla*	0	0	1	2	0	3
	T. mossambica	1	0	0	1	0	2
Liver	*C. catla*	1	1	0	2	0	4
	T. mossambica	2	0	0	1	0	3
Intestine	*C. catla*	1	2	0	1	0	4
	T. mossambica	1	0	1	1	0	3
Muscle	*C. catla*	0	0	2	0	0	2
	T. mossambica	0	1	1	0	0	2
Brain	*C. catla*	0	0	2	0	1	3

	T. mossambica	1	1	0	0	0	2
Eye	*C. catla*	2	0	0	0	0	2
	T. mossambica	0	2	0	0	0	2

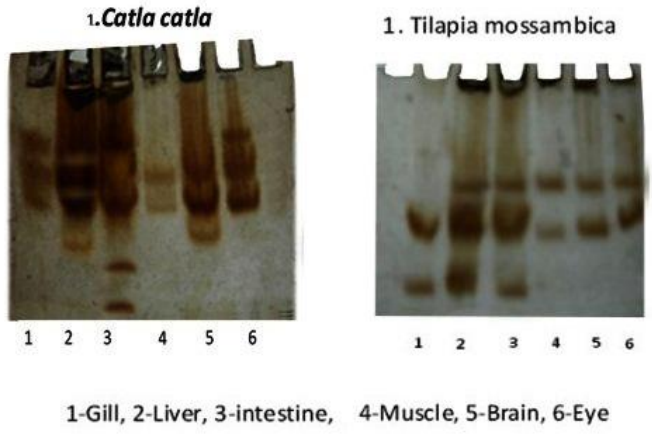

Fig 3. Figure of esterase zones in *Catla Catla* and *Tilapia Mossambica*

3.6 Summary of Key Differences

Table 12. Major differences in esterase patterns between *C. catla and T. mossambica*

Feature	*Catla catla*	*Tilapia mossambica*
Total zones	6	3
Rm range	0.96 – 0.33	0.96 – 0.66
Fastest zone	0.96 (ER)	0.96 (CE)
Slowest zone	0.33 (CE/ChE/CHsp)	0.66 (ER/ChE)
Predominant class	ER (40% of zones)	CE (41% of zones)
ArE presence	Present (brain)	Absent
CHsp presence	Present (multiple tissues)	Present (limited)
Tissue with most zones	Liver, Intestine (4 each)	Liver, Intestine (3 each)
Eye esterases	CE only	ChE only

4. DISCUSSION

4.1 Tissue Specificity of Esterase Patterns

The present study demonstrates highly tissue-specific esterase patterns in both Catla catla and Tilapia mossambica, confirming earlier observations in diverse fish species. Each tissue exhibited a characteristic electrophoretic profile reflecting its physiological functions and metabolic requirements. These findings align with the extensive documentation of tissue-specific esterase distribution in fishes by Holmes et al. (1968), Hart and Cook (1976), and Varma and Frankel (1980).

In *C. catla,* liver and intestine displayed the highest number of esterase zones (four each), consistent with their roles as primary metabolic and digestive organs. Simonarson and Watts (1969) similarly reported liver and intestine as rich sources of esterolytic activity in marine and freshwater fishes. The presence of multiple esterase forms in these tissues likely reflects their involvement in lipid metabolism, detoxification of xenobiotics, and processing of dietary esters (Masters and Holmes, 1975).

The brain of *C. catla* exhibited three zones, including a unique arylesterase (ArE) at Rm 0.56 not found in other tissues. This tissue-specific expression of ArE in brain tissue has been reported in several Barbus species by Varma and Frankel (1980) and in perciform fishes by Rajaiah (1996). The functional significance of brain-specific ArE remains unclear but may relate to neurotransmitter metabolism or protection against oxidative stress.

Eye tissue in both species showed relatively simple patterns (two zones each), consistent with observations by Haritos and Salamastrakis (1982) in Trachurus species. However, the esterase classes differed markedly—C. catla eye exhibited exclusively CE, while T. mossambica eye showed exclusively ChE. This fundamental difference may reflect variations in retinal physiology or cholinergic transmission requirements between cyprinids and cichlids.

4.2 Species Specificity and Taxonomic Implications

The distinct esterase patterns observed between C. catla and T. mossambica support the utility of these enzymes as biochemical markers for species identification. The two species share no common zones with identical Rm values and inhibitor sensitivity, despite both inhabiting similar freshwater environments. This complete divergence in esterase profiles underscores the evolutionary distance between Cypriniformes and Perciformes orders.

C. catla exhibited greater esterase multiplicity (6 zones) compared to *T. mossambica* (3 zones). Similar variations in esterase complexity among fish species have been reported by numerous investigators. Simonarson and Watts (1969) found that marine fishes generally displayed wider esterase diversity than freshwater species. However, both species in the present study are freshwater inhabitants, suggesting that phylogenetic position rather than habitat primarily determines esterase multiplicity.

The presence of arylesterase (ArE) exclusively in C. catla brain and its absence in all T. mossambica tissues represents a clear species-specific marker. Varma and Frankel (1980) reported similar species-specific distribution of ArE among four Barbus species, with some species expressing ArE in liver while others lacked it entirely. Such qualitative differences provide reliable taxonomic characters for species discrimination.

The zone with Rm 0.75 in *T. mossambica* exhibited remarkable tissue-dependent variation in inhibitor sensitivity, appearing as CE in liver and brain, CHsp in muscle and intestine, ER in gill, and ChE in eye. This phenomenon, where electrophoretically identical zones display different catalytic properties in different tissues, has been documented by Hart and Cook (1976) and Swapna (2024) in Brachydanio species and attributed to post-translational modifications or tissue-specific subunit associations.

5. CONCLUSION

The comparative analysis of tissue esterase polymorphism in *Catla catla* and *Tilapia mossambica* reveals clear species- and tissue-specific patterns reflecting phylogenetic divergence and functional adaptation. C. catla (Cypriniformes) showed higher

esterase multiplicity with six zones, while *T. mossambica* (Perciformes) exhibited only three, indicating evolutionary differences at the biochemical level. Tissue distribution patterns demonstrated maximal activity in liver and intestine, minimal in muscle and eye, and a unique arylesterase in the brain of *C. catla*. Notably, the Rm 0.75 zone in *T. mossambica* displayed tissue-dependent inhibitor sensitivity, indicating enzymatic plasticity. Esterase-resistant (ER) forms predominated in gill tissues of both species, suggesting adaptation to environmental exposure, while cholinesterases were abundant in excitable tissues, supporting their role in neurotransmission. The absence of shared zones and qualitative differences confirm esterase zymograms as reliable taxonomic markers and potential biomarkers for monitoring pesticide exposure in freshwater ecosystems.

Competing interests:

The authors declare that they have no competing interests

References

[1] Abdur Rashid, Md. (2012). Esterase banding pattern in different age groups of Mosquito fish, Gambusia affinis in Bangladesh. International Journal of Environmental Biology, 2(3): 162-164.

[2] Abdur Rashid, Md. and Dil Amena Habib. (2012). Tissue specific esterases isozymes banding pattern in Silver carp (Hypophthalmicthyes molitrix) at different developmental stages. International Journal of Research in Zoology, 2(2): 18-22.

[3] Ahasan, M.M., Rahman, M.A. and Jamal Uddin, A.F.M. (2012). Genetic polymorphism based on β-naphthyl acetate in different age group of Gambusia affinis in Bangladesh. Journal of the Bangladesh Academy of Sciences, 36(1): 39-44.

[4] Aldridge, W.N. (1953). Serum esterases: two types of esterases (A and B) hydrolyzing p-nitrophenyl acetate, propionate and butyrate and a method for their determination. Biochemical Journal, 53: 110-117.

[5] Amita Saxena and Soranganba, N. (2012). Esterases diversity in Indian and exotic carps. Journal of Experimental Zoology India, 15(2): 451-456.

[6] Augustinson, K.B. (1959). Electrophoretic studies on blood plasma esterases-II. Avian, reptilian and piscine plasmata. Acta Chemica Scandinavica, 13: 1081-1096.

[7] Augustinson, K.B. (1960). Butyryl and propionyl cholinesterases and related types of eserine-sensitive esterases. In: The Enzymes (Boyer, P.D., Lardy, H. and Myrback, K. Eds.), 4: 521-540. Academic Press, New York.

[8] Augustinson, K.B. (1961). Multiple forms of esterase in vertebrate blood plasma. Annals of the New York Academy of Sciences, 94: 844-860.

[9] Begum, R.A., Islam, A., Shahjahan, R.M. and Abdur Rashid, Md. (2012). Tissue specific and comparative esterase variability in Notopterus notopterus and Notopterus chitala (Osteoglossiformes: Notopteridae). International Journal of Research in Fisheries and Aquaculture, 2(4): 44-47.

[10] Begum, R.A., Rahman, D.T., Abdul Rashid, Md., Alam, M.S. and Shahjahan, R.M. (2012). Comparison of esterases isozyme variability in some selected tissues of the Asian and African catfishes (Siluriformes: Clariidae). Bangladesh Journal of Zoology, 40(1): 43-50.

[11] Begum, R.A., Shahjahan, R.M., Nur, F.M., Rahman, H. and Kabir, M.A. (2010). Analysis of esterase isozyme banding in some tissues of Nile tilapia and Genetically Improved Farmed Tilapia Oreochromis niloticus L. Bangladesh Journal of Zoology, 38: 119-126.

[12] Bergmann, F., Segal, R. and Rimon, S. (1957). A new type of esterase in hog kidney extract. Biochemical Journal, 67: 481-486.

[13] Bornscheuer, U.T. (2002). Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiology Reviews, 26: 73-81.

[14] Bornscheuer, U.T., Bessler, C., Srinivas, R. and Krishna, S.H. (2002). Optimizing lipases and related enzymes for efficient application. Trends in Biotechnology, 20(10): 433-437.

[15] Chakraborty, S.K. (1989). Electrophoretic study of muscle and eye lens proteins of three species of Nemipterids. In: Fish Genetics in India (Das, P. and Jhingran, A.G. Eds.), pp. 115-118.

[16] Chatterjee, K. (1989). Cytotaxonomic and electrophoretic investigation on Indian air breathing fishes. In: Fish Genetics in India (Das, P. and Jhingran, A.G. Eds.), pp. 83-99.

[17] Ecobichon, D.J. and Israel, Y. (1967). Characterization of the esterases from electric tissue of Electrophorus by starch gel electrophoresis. Canadian Journal of Biochemistry, 45: 1099-1105.

[18] Holmes, R.S. and Whitt, G.S. (1970). Developmental genetics of the esterase isozymes of Fundulus heteroclitus. Biochemical Genetics, 4: 471-480.

[19] Knowles, C.O., Arurkar, S. and Hogan, J. (1968). Electrophoretic separation of fish brain esterases. Journal of the Fisheries Research Board of Canada, 25: 1517-1519.

[20] Koehn, R.K. (1969). Esterase heterogeneity: Dynamics of a polymorphism. Science, 163: 943-944.

[21] Koehn, R.K. (1970). Functional and evolutionary dynamics of polymorphic esterases in catostomid fishes. Transactions of the American Fisheries Society, 99: 219-228.

[22] Koehn, R.K. and Johnson, D.W. (1967). Serum transferrin and serum esterase polymorphisms in an introduced population of the bigmouth buffalo fish Ictiobus cyprinellus. Copeia, 4: 805-808.

[23] Lakshmipathi, V. (1994). Electrophoretic separation of proteins. In: Analytical Instrumentation and Techniques (Bhagava Raju, I.V.K. et al. Eds.), Central Instrumentation Centre, Kakatiya University, Warangal.

[24] Lakshmipathi, V. and Reddy, T.M. (1989). Esterase polymorphism in muscle and brain of four fresh water fishes belonging to the family Cyprinidae. Journal of Applied Ichthyology, 5: 88-95.

[25] Lakshmipathi, V. and Reddy, T.M. (1990). Comparative study of esterases in brain of the vertebrates. Brain Research, 521: 321-324.

[26] Masters, C.J. and Holmes, R.S. (1974). Isozymes, multiple enzyme forms, and phylogeny. Advances in Comparative Physiology and Biochemistry, 5: 109-195.

[27] Masters, C.J. and Holmes, R.S. (1975). Haemoglobin, Isozymes and Tissue Differentiation. Frontiers of Biology Series, Vol. 42. North-Holland Publishing Co., Amsterdam.

[28] Metcalf, R.A., Whitt, G.S. and Childers, W.F. (1972). Inheritance of esterases in the white crappie (Pomoxis annularis), black crappie (P. nigromaculatus) and their F_1 & F_2 interspecific hybrids. Animal Blood Groups and Biochemical Genetics, 3: 19-33.

[29] Nyman, I. (1965). Inter and intraspecific variations of proteins in fishes. Annals Academiae Regiae Scientiarum Upsaliensis, 9: 1-18.

[30] Rajaiah, V. (1996). Tissue and species specific distribution of esterases in some selected fresh water fishes of Warangal District. Ph.D. Thesis, Kakatiya University, Warangal.

[31] Swapna, K., Srujana, M., & Mamidala, E. (2024). Identification of steroidal cardenolides from Calotropis procera as novel HIV-1 PR inhibitors: A molecular docking & molecular dynamics simulation study. The Indian journal of medical research, 160(1), 78.

From National Conference on Advances in Life Sciences: Present & Future (NCALS–2026) | 24-25 March 2026 | Organized by: Department of Zoology, Kakatiya University, Warangal-506 009, Telangana State, India