

Tissue-Specific Esterase Isozyme Patterns in *Oreochromis mossambicus* (cypriniformes) and *Puntius sarana* (perciformes) Revealed by Zymography and Inhibitor Profiling

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Abstract: Tissue-specific esterase isozyme profiles were investigated in gill, liver, intestine, muscle, brain, and eye tissues of *Oreochromis mossambicus* and *Puntius sarana* using electrophoretic zymography and inhibitor-based classification. In *O. mossambicus*, three esterase zones with relative mobility (Rm) values of 0.96, 0.75, and 0.66 were detected. The Rm 0.96 zone occurred in gill, liver, and intestine and was classified as carboxylesterase (CE). The Rm 0.75 zone was present in all tissues but showed tissue-dependent classification, including CE, esterase resistant to inhibitors (ER), cholinesterase (ChE), and CHsp esterase. The Rm 0.66 zone was present in all tissues except gill and was classified mainly as ER or ChE esterase. In *P. sarana*, five zones with Rm values of 0.86, 0.75, 0.66, 0.50, and 0.33 were identified. Gill, liver, and intestine showed four esterase zones each, whereas muscle, brain, and eye showed three zones. The Rm 0.50 and 0.33 zones were ubiquitous in *P. sarana*, while the Rm 0.86 zone was restricted to intestine. ER esterases predominated in gill, liver, intestine, and brain, whereas ChE and CE esterases displayed marked tissue specificity. These findings demonstrate clear interspecific and tissue-dependent heterogeneity in esterase isozyme expression, supporting the value of zymographic inhibitor profiling for biochemical characterization and environmental biomonitoring of freshwater fishes.

Keywords: Esterase Isozymes; Zymography; Carboxylesterase; Cholinesterase; Tissue Specificity; *Oreochromis mossambicus*; *Puntius sarana*.

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I. INTRODUCTION

Electrophoresis is a key technique for studying protein and enzyme variation among tissues and species by separating molecules according to charge and size. Acrylamide gel electrophoresis is especially useful because gel porosity can be adjusted to improve resolution. Esterase banding patterns are widely used to identify species, from microbes to vertebrates, and to resolve taxonomic problems (Masters and Holmes, 1975). However, the extensive multiplicity and heterogeneity of esterases make complete enzyme characterization difficult.

Mid-twentieth-century advances in comparative biochemistry showed that biochemical diversity parallels biological diversity and can help solve species problems (Anfinsen, 1959; Dixon, 1966; Florkins, 1966; Thoai and Roche, 1968; Watts, 1968; Schoffeniels, 1971). This led to extensive use of biochemical markers, especially tissue-specific proteins and enzymes, in taxonomy (Wright, 1974; Turner, 1984; Whitt, 1987). Gel electrophoresis has

provided valuable data on protein and enzyme variation within and between species (Utter, 1985) and is now widely applied in population genetics, systematics, and evolution (Gottlieb, 1971; Guttman, 1973; Avise, 1974; Nevo, 1978; Buth, 1984; Utter, 1985). Combined with histochemical staining (Hunter and Markert, 1957; Shaw and Prasad, 1970; Selander et al., 1971; Smith, 1976), individual enzymes can be precisely located on gels, revealing tissue- and species-specific patterns. Many enzymes now serve as biochemical markers for species and stock identification (Ayala, 1983; Utter, 1985).

Numerous electrophoretic studies have examined fish protein and enzyme variation (Utter et al., 1974; O'Rourke, 1974; Kirpichnikov, 1981; Ayala, 1983; Turner, 1984; Ryman and Utter, 1987; Whitt, 1987). Despite this progress, electrophoretic work on Indian fishes remains limited, with only a few groups making notable contributions.

Earlier Indian contributions on esterase polymorphisms include Chandrashekar (1959), Das (1961),

Hussain and Siddiqui (1974), Menezes (1975, 1980), Menezes and Qasim (1984), Krishnaja and Rege (1977), Triveni (1980, 1984), Basu et al. (1981), Dhar and Chatterjee (1984), Chatterjee (1989), Joseph et al. (1985), Chakraborty (1989), Rao et al. (1989), Padhi and Khuda Baksh (1990), Menezes et al. (1992, 1993), Patwardhan and Modak (1992), and Pandey and Hasnain (1994). Reports from Pakistan include Malik and Noor (1993) and Malik and Anjum (1993).

Although these studies have revealed useful electrophoretic differences and genetic relationships in Indian fishes (Rao et al., 1989; Patwardhan and Modak, 1992; Menezes et al., 1993), they cover only a small fraction of the vast fish diversity in India. The present study focuses on tissue-specific esterase isozyme patterns in *Oreochromis mossambicus* and *Puntius sarana* using zymography and inhibitor profiling, contributing to the biochemical characterization and potential environmental biomonitoring of freshwater fishes.

II. ESTERASES

Esterases hydrolyze esters into an acid and an alcohol. Lovenhart (1906) first recognized two categories: esterases acting on short-chain (C2–C4) fatty acid esters and lipases acting on long-chain (>C8) fatty acid esters (Seligman and Nachlas, 1950). These enzymes participate in nerve impulse control, reproduction, development, detoxification, and xenobiotic tolerance, and serve as biomarkers of environmental pollution and gene markers in many organisms. They are also used in food, detergent, fine chemical, wastewater treatment, biodiesel, pharmaceutical, and bioremediation industries (Rao et al., 1998; Sharma et al., 2001; Bornscheuer et al., 2002; Jaeger and Eggert, 2002; Reetz, 2002; Maurer, 2004; Cammarota and Freire, 2006; Hasan et al., 2006). Their high regio- and stereo-specificity supports kinetic resolution of optical isomers for optically pure compounds (Bornscheuer, 2002; Hasan et al., 2006), and their cofactor-independent catalysis is advantageous (Bornscheuer, 2002). Esterases also play a vital role in insect metamorphosis (Quan-You Yu et al., 2009).

Organophosphate inhibition was a major advance in esterase research (Webb, 1948). Aldridge (1953) used E-600 (diethyl-p-nitrophenyl phosphate) to distinguish 'A' esterases (hydrolyze the inhibitor) from 'B' esterases (sensitive to it). A third type, 'C' esterase (Bergman et al., 1957), was neither sensitive to nor capable of hydrolyzing organophosphates and was slightly activated by low pCMB (parachloromercuribenzoate). Early non-specific esterase studies produced varied nomenclature, but these were essentially synonyms of A, B, and C esterases (Pearse, 1972):

- A-Esterase = Aromesterase = Arylesterase – Organophosphate-resistant esterase = E-600 esterase = DFPase.
- B-Esterase = Aliesterase – Carboxylesterases = Organophosphate-sensitive esterase.

- C-Esterase = Acetylerase = Organophosphate-resistant, sulphydryl-resistant esterase.

Cholinesterases were distinguished by eserine (physostigmine) sensitivity (Myers and Mendel, 1949). Substrate-specificity classification (Tashian, 1965; Schiff, 1970) proved inadequate because many forms show broad specificity (Hart and Cook, 1976; Varma and Frankel, 1980; Lakshmipathi and Reddy, 1989). Inhibitor sensitivity enabled four categories (Augustinson, 1961; Holmes and Masters, 1967; Holmes et al., 1968):

- Carboxylesterases (E.C. 3.1.1.1) – Inhibited by organophosphates but not eserine; prefer short-chain aliphatic and aromatic esters (Webb, 1948; Aldridge, 1953; Hofstee, 1960; Augustinson, 1961; Holmes and Masters, 1967).
- Arylesterases (E.C. 3.1.1.2) – Inhibited by pCMB and pHMB; prefer aromatic esters, especially acetic esters (Aldridge, 1953; Mounter, 1960; Main, 1960; Augustinson, 1961; Holmes and Masters, 1967).
- Acetylerases (E.C. 3.1.1.6) – Resistant to organophosphates, physostigmine, and pCMB/pHMB (Bergman et al., 1957; Bergman and Rimon, 1960; Holmes and Masters, 1967).
- Cholinesterases – Include acetylcholinesterases (AChE, E.C. 3.1.1.7) and pseudocholinesterases (E.C. 3.1.1.8); inhibited by organophosphates and eserine. AChE prefers acetylcholine and aromatic acetyl esters (Richter and Croft, 1942; Wilson, 1960; Froedi and Wilson, 1971), while pseudocholinesterases hydrolyze butyrylcholine and aromatic butyrate esters (Augustinson, 1960; Janz and Cohen, 1962; Harris, 1970).

This scheme was successfully applied to vertebrate tissue esterases (Augustinson, 1961; Holmes and Masters, 1967; Holmes et al., 1968). Later fish studies (Hart and Cook, 1976; Varma and Frankel, 1980; Haritos and Salamastrakis, 1982; Lakshmipathi and Reddy, 1989) found it insufficient, adding four more classes:

- ER esterases – Resistant to OP compounds, physostigmine, and pCMB/pHMB, but without preference for acetate esters (unlike acetylerases).
- Esdp esterases – Inhibited by OP compounds and pCMB/pHMB.
- Ese esterases – Inhibited by physostigmine (eserine) alone.
- CHsp esterases – Cholinesterase-like enzymes inhibited by OP compounds, physostigmine, and pCMB/pHMB.

➤ Heterogeneity and Multiplicity of Esterases

Esterases show greater heterogeneity and multiplicity than most other enzymes; a single tissue may have 30 or more heteromorphs (Masters and Holmes, 1975; Holmes et al., 1968). Homologous tissues of different species, even closely related ones, vary widely in heterogeneity and multiple enzyme forms (Masters and Holmes, 1975). Electrophoresis helped reveal this variation; comparing

relative mobilities of zones within and between tissues and species, along with substrate specificity and inhibitor sensitivity, allowed tissue- and species-specific comparisons across animal groups (Masters and Holmes, 1974, 1975). Though the significance of esterase multiplicity is unclear, its widespread occurrence suggests a multi-component system serving physiological needs (Masters and Holmes, 1974). Esterases are used to distinguish vertebrates (Masters and Holmes, 1975; Ahuja et al., 1977), invertebrates (Manwell et al., 1967; Freyvogel et al., 1968; Reid and Dunnill, 1969; Oien and Stenersen, 1984; Sujatha, 1991), and microbes (Norris and Burges, 1963), and to identify tissue differentiation stages during development (Holmes and Whitt, 1970; Lakshmipathi and Sujatha, 1991; Shobharani, 1993; Shobharani and Lakshmipathi, 1995). Their use as cytochemical markers (Shnitka and Seligman, 1961) and binding to lipids and lipoproteins during hydrolysis and transport (Okuda and Fuji, 1968) suggest that multiplicity confers adaptive advantage (Masters and Holmes, 1974).

Tissue esterase studies began in the late 1960s. Ecobichon and Israel (1967) classified electric tissue esterases into acetylcholinesterases, pseudocholinesterases, and carboxylesterases. Hogan and Knowles (1968) described brain acetylcholinesterases in channel catfish and bluegill as potential biomonitors of organophosphate insecticides. Knowles et al. (1968) classified brain esterases of these fishes using organophosphates and eserine. Holmes et al. (1968) characterized esterases in several vertebrates, including catfish *Cnidoglanis megastomus*, finding no cholinesterases. Pantelouris and Payne (1968) studied eel esterase genetics. Simonarson and Watts (1969) found greater esterase divergence in marine than freshwater fishes and differences among herring stocks, using NEM, pCMB, pMSF, and physostigmine salicylate. McCabe and Dean (1970) classified skipjack tuna esterases as carboxylesterases and subdivided acetyl esterases.

Hart and Cook (1976) compared tissue esterases of *Brachydanio rerio* and *B. albolineatus*, finding 10–19 zones with tissue- and species-specific patterns, and introduced ER, ESE, and ESDP classes. Hart and Cook (1977) reported tissue-specific esterase appearance during development and high maternal contribution in hybrids. Ahuja et al. (1977) found esterases more tissue- than species-specific in two Xiphophorus fishes and their hybrids. Hodges and Whitmore (1977) reported carboxylesterase polymorphism in *Gambusia affinis* muscle. Gaudie et al. (1977) found similar esterase patterns in two *Trachurus* species. Varma and Frankel (1980) compared liver and intestinal esterases in four *Barbus* species, finding distinct tissue patterns and similar properties among geographically related species, using Hart and Cook's (1976) classification. Haritos and Salamastrakis (1982) found species-specific esterase bands in three *Trachurus* species. Grigorena and Rakic (1985) studied AChE specificity in electric ray.

The present study extends these investigations by examining tissue-specific esterase isozyme patterns in *Oreochromis mossambicus* and *Puntius sarana* using

electrophoretic zymography and inhibitor-based classification. Six tissues (gill, liver, intestine, muscle, brain, and eye) were analyzed to detect tissue-specific distribution and classify individual esterase zones based on their sensitivity to pCMB, eserine, and paraoxon. The patterns represent averages of multiple individuals per species, repeated for reproducibility, with individuals collected from a single locality to avoid inter-population variation. The findings aim to provide baseline biochemical markers for species identification, physiological studies, and monitoring of pesticide or xenobiotic exposure in freshwater ecosystems.

III. RESULTS

➤ *Oreochromis mossambicus*

• Gill

The gill tissue resolved into two esterase fractions on the zymogram, with relative mobility (R_m) values of 0.96 and 0.75, both displaying moderate staining intensity. Of these two bands, the fraction at R_m 0.75 was categorized as an ER esterase, while the band at R_m 0.96 was identified as a carboxylesterase (CE).

• Liver

Three active esterase zones were detected in the liver, with R_m values of 0.96, 0.75, and 0.66. The fractions at R_m 0.96 and 0.75 were both classified as carboxylesterases (CE), exhibiting relatively higher enzymatic activity. The remaining band at R_m 0.66 corresponded to an ER esterase with moderate activity.

• Intestine

The intestinal zymogram revealed three esterase bands with R_m values of 0.96, 0.75, and 0.66. The fraction at R_m 0.75 was identified as a CHsp esterase showing higher activity, whereas the bands at R_m 0.96 and 0.66 were characterized as CE and ER esterases, respectively, both with moderate activity.

• Muscle

The muscle tissue displayed only two esterase zones, with R_m values of 0.75 and 0.66, both exhibiting moderate activity. The band at R_m 0.75 was classified as a CHsp esterase, while the fraction at R_m 0.66 was designated as a cholinesterase (ChE).

• Brain

Two esterase bands were observed in the brain tissue, with R_m values of 0.75 and 0.66. The fraction at R_m 0.75 was identified as a carboxylesterase (CE), whereas the band at R_m 0.66 was classified as a cholinesterase (ChE), both showing moderate activity.

• Eye

The eye tissue presented two active esterase fractions on the zymogram, with R_m values of 0.75 and 0.66, both displaying moderate activity. Both bands were inhibited by paraoxon and eserine, and were therefore categorized as cholinesterases (ChE).

Only three different zones were found in the tissue-specific distribution of esterases in *Oreochromis mossambicus*, with Rm values of 0.96, 0.75, and 0.66. Among these, the proportion at Rm 0.96 was found in the colon, liver, and gills, where it was associated with carboxylesterases (CE). The band at Rm 0.75 was found in every tissue tested; it manifested as an ER esterase in the

gill, a cholinesterase (ChE) in the eye, a carboxylesterase (CE) in the liver and brain, and a CHsp esterase in the muscle and gut. Additionally, the proportion at Rm 0.66 was seen in five tissues, showing up as an ER esterase in the gut and liver (but not in the gills) and as a cholinesterase (ChE) in the muscle, brain and eye.

Table 1 Inhibitor Sensitivity of Individual Esterase Zones in *Oreochromis mossambicus*

Name of Tissue	Gill		Liver			Intestine			Muscle		Brain		Eye	
Rm values	.96	.75	.96	.75	.66	.96	.75	.66	.75	.66	.75	.66	.75	.66
Activity	+	++	++	+++	++	+	+++	++	+	+	+++	++	++	+
pCMB	+	+	+	+	+	+	-	+	-	+	+	+	+	+
Eserine	+	+	+	+	+	+	-	+	-	-	+	-	-	-
Paraoxon	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Classification	CE	ER	CE	CE	ER	CE	CHsp	ER	CHsp	ChE	CE	ChE	ChE	ChE

+++ = High Activity; ++ = Moderate Activity; += Low Activity; + = Very Low Activity;

- ✓ Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.
- ✓ CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;
- ✓ Esdp= Esterase sensitive to organophosphates and pCMB
- ✓ Ese = Esterases sensitive to eserine alone;

Table 2 Tissue Specific Distribution of Esterases in *Oreochromis mossambicus*

Rm values	1	2	3
Tissues	.96	.75	.66
1) Gill	+	+	
	CE	ER	
2) Liver	++	+++	++
	CE	CE	ER
3) Intestine	+	+++	++
	CE	CHsp	ER
4) Muscle		+	+
		CHsp	ChE
5) Brain		+++	++
		CE	ChE
6) Eye		++	+
		ChE	ChE

+++ = High Activity; ++ = Moderate Activity; += Low Activity; + = Very Low Activity;

- ✓ Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.
- ✓ CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;
- ✓ Esdp = Esterase sensitive to organophosphates and pCMB
- ✓ Ese = Esterases sensitive to eserine alone;

➤ *Tissue Specific Distribution of Esterases in Oreochromis mossambicus*

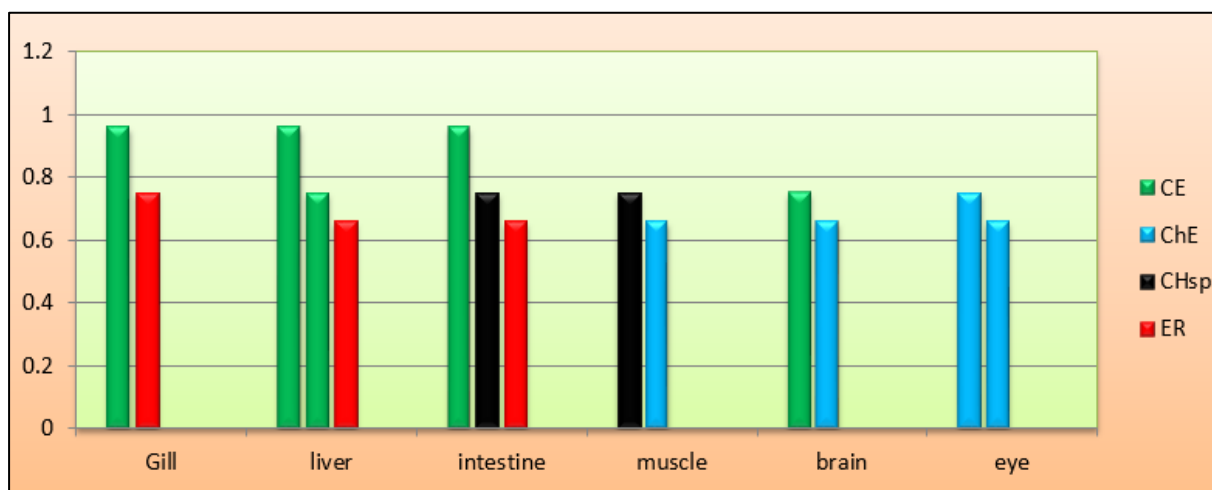


Fig 1 Tissue Specific Distribution of Esterases in *Oreochromis mossambicus*

➤ *Puntius sarana* (F.Hamilton 1822)

• *Gill*

Zymogram analysis of gill tissue of *Puntius sarana* revealed four bands for esterases with Rm values 0.75, 0.66, 0.50, and 0.33. Band at Rm 0.50 was sensitive to paraoxon and eserine and was considered as cholinesterase (ChE). Bands at Rm 0.75 and 0.66 were characterized as ER esterases, while fraction at Rm 0.33 was classified as CHsp esterase.

• *Liver*

Zymogram analysis of the liver tissue showed four esterase bands with Rm values of 0.75, 0.66, 0.50, and 0.33. Fractions at Rm 0.75 and 0.66 remained insensitive to any inhibitor and were designated as ER esterases. Band at Rm 0.33 was inhibited by paraoxon and eserine and was classified as cholinesterase (ChE). The rest fraction at Rm 0.50 was characterized as carboxylesterase (CE).

• *Intestine*

Zymogram analysis of the intestine showed four active esterase fractions with Rm values of 0.86, 0.66, 0.50, and 0.33. Fractions at Rm 0.86 and 0.66 were characterized as ER and Esdp esterases, respectively. Bands at Rm 0.50 and 0.33 were inhibited by paraoxon and eserine and were classified as cholinesterases (ChE). All fractions were characterized by relatively high activity, except for the fraction at Rm 0.86.

• *Muscle*

Three esterase fractions with Rm values 0.66, 0.50, and 0.33 were detected in muscle tissue. The fractions with Rm values 0.50 and 0.66 were determined to be CHsp esterases, whereas the fraction with Rm value 0.33 was determined to be carboxylesterase (CE). All three fractions were moderately active.

• *Brain*

The brain tissue demonstrated three bands of esterase, having Rm values of 0.75, 0.50, and 0.33. They were

determined to be ER, Esdp, and arylesterase (ArE) and all had moderate activity.

• *Eye*

In eye tissue, three bands of esterase were observed on zymogram, having Rm values of 0.75, 0.50, and 0.33, all having moderate activity. The band having Rm value 0.75 was found to be a CHsp esterase, the band having Rm value 0.50 was found to be a carboxylesterase (CE) and the band having Rm value 0.33 was found to be cholinesterase (ChE).

Based on the relative mobilities, the esterase fractions found among different tissues of *Puntius sarana* were categorized into five unique zones, with Rm values of 0.86, 0.75, 0.66, 0.50, and 0.33. Different tissues had their unique esterase zones. The esterase fractions at Rm 0.50 and 0.33 were found in all the tissues analyzed. The esterase at Rm 0.50 was found to be a cholinesterase (ChE) in the gill and intestine; a carboxylesterase (CE) in the liver and eye; and Esdp and CHsp esterases in the brain and muscle, respectively. The esterase at Rm 0.33 was found to be a cholinesterase (ChE) in the liver, intestine, and eye; CHsp, ArE, and CE esterases in the gill, brain, and muscle, respectively. The esterase bands at Rm 0.75 and 0.66 were found in four tissues each. The esterase at Rm 0.75 was found to be an ER esterase in the gill, liver, and brain; but a CHsp esterase in the eye. The esterase at Rm 0.66 was found to be an ER esterase in the gill and liver; while Esdp and CHsp esterases in the intestine and muscle, respectively. The esterase at Rm 0.86 was found to be the fastest moving zone and Taking into account the Rm values of the esterase fractions detected in tissues of *Puntius sarana*, it is possible to classify them into five different zones with Rm values of 0.86, 0.75, 0.66, 0.50, and 0.33. Each tissue demonstrated a distinct banding pattern. Of all the different types of tissues studied, there were four esterase bands found in the gill, liver, and intestine tissues, while the other tissues had three bands each. When looking at the type of esterases present, it is worth noting that ER esterases were the most common in almost all the tissues, followed by ChE. Only one ArE was found in the brain tissue.

Table 3 Inhibitor Sensitivity of Individual Esterase Zones in *Puntius sarana*

Name of Tissue	Gill				Liver				Intestine				Muscle			Brain			Eye		
Rm values	.75	.66	.50	.33	.75	.66	.50	.33	.86	.66	.50	.33	.66	.50	.33	.75	.50	.33	.75	.50	.33
Activity	+	+	++	++	+	++	++	++	+	++	++	++	++	++	+	+	++	++	++	+	++
pCMB	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	+	+
Eserine	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	+	+	+	-	+	-
Paraoxon	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-
Classification	ER	ER	ChE	CHsp	ER	ER	CE	ChE	ER	Esdp	ChE	ChE	CHsp	CHsp	CE	ER	Esdp	ArE	CHsp	CE	ChE

+++ = High Activity; ++ = Moderate Activity; += Low Activity; + = Very Low Activity;

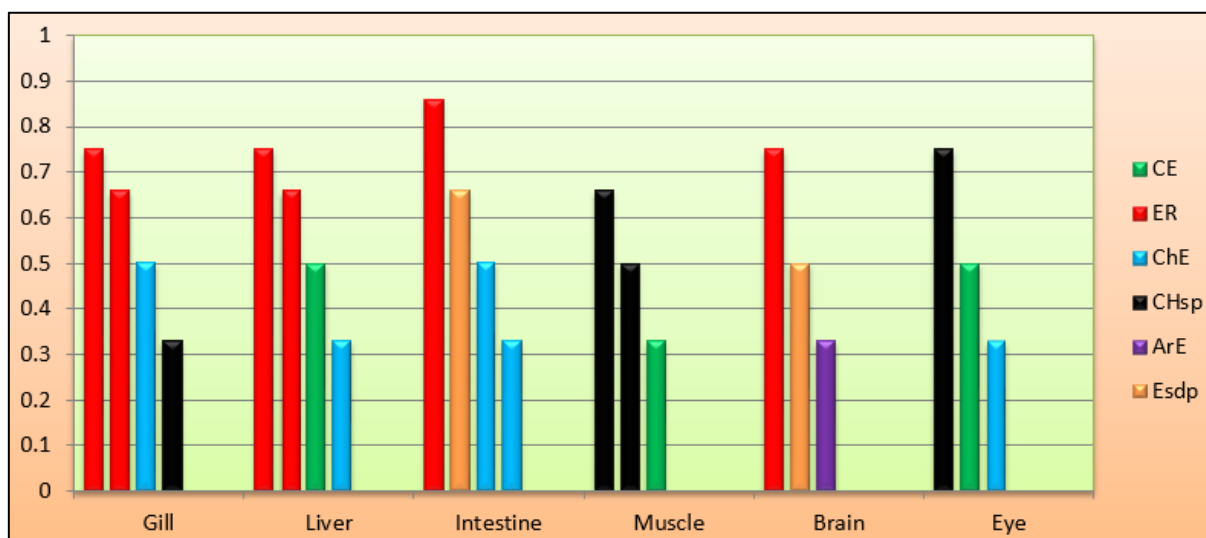
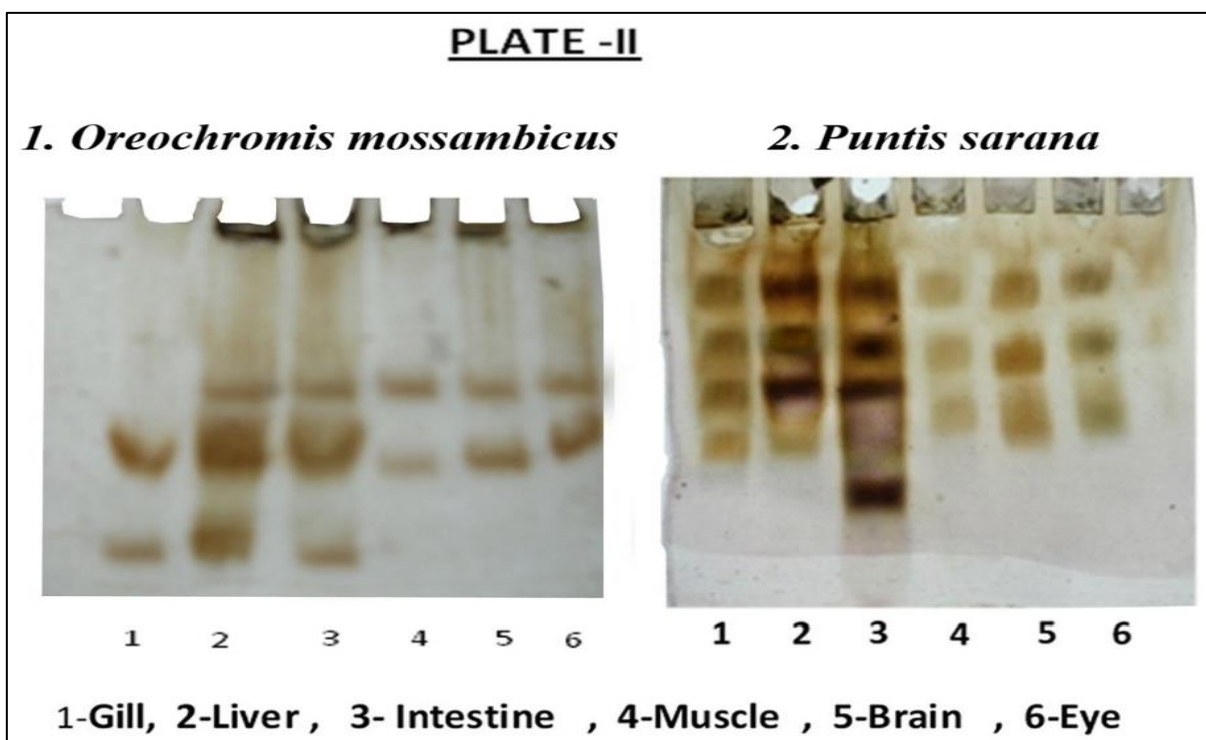
- ✓ Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.
- ✓ CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;
- ✓ Esdp = Esterase sensitive to organophosphates and pCMB
- ✓ Ese = Esterases sensitive to eserine alone;

Table 4 Tissue Specific Distribution of Esterases in *Puntius sarana*

Rm values	1	2	3	4	5
Tissues	.86	.75	.66	.50	.33
1) Gill		++ ER	++ ER	++ ChE	++ CHsp
2) Liver		++ ER	+++ ER	+++ CE	++ ChE
3) Intestine	++ ER		+++ Esdp	+++ ChE	+++ ChE
4) Muscle			++ CHsp	++ CHsp	++ CE
5) Brain		++ ER		++ Esdp	++ ArE
6) Eye		++ CHsp		++ CE	++ ChE

+++ = High Activity; ++ = Moderate Activity; += Low Activity; + = Very Low Activity;

- ✓ Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.
- ✓ CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;
- ✓ Esdp = Esterase sensitive to organophosphates and pCMB
- ✓ Ese = Esterases sensitive to eserine alone;

➤ Tissue Specific Distribution of Esterases in *Puntius sarana*Fig 2 Tissue Specific Distribution of Esterases in *Puntius sarana*Fig 3 *Oreochromis mossambicus* and *Puntius sarana***IV. DISCUSSION**

The present findings reveal strong tissue-specific and species-specific variation in esterase isozyme patterns in *O. mossambicus* and *P. sarana*. This heterogeneity is expected because esterases comprise a broad group of enzymes with different physiological roles, substrate preferences, cellular locations, and sensitivities to inhibitors.

In *O. mossambicus*, the three observed mobility zones indicate a comparatively simple esterase profile. The occurrence of the Rm 0.96 CE zone in gill, liver, and intestine is biologically meaningful because these tissues are

important interfaces for chemical uptake, metabolism, and detoxification. Gills are in direct contact with the aquatic environment, whereas the intestine is exposed to dietary contaminants. The liver is a principal metabolic and detoxifying organ and is commonly enriched in carboxylesterase activity. The high activity of CE zones in liver supports a possible role in hydrolysis and detoxification of ester-containing xenobiotics.

The Rm 0.75 zone was present in all tissues of *O. mossambicus* but differed in esterase class. This pattern suggests that identical electrophoretic mobility does not necessarily indicate the same enzyme across tissues.

Isozymes with similar migration may differ in inhibitor sensitivity, substrate specificity, molecular structure, or post-translational modification. Therefore, classification should be interpreted as operational—based on the inhibitor-response profile—rather than as definitive molecular identification.

The eye of *O. mossambicus* showed two ChE zones, both inhibited by paraoxon and eserine. Cholinesterases are particularly relevant in neural and sensory tissues because they regulate cholinergic neurotransmission. The detection of ChE activity in brain, muscle, and eye is therefore consistent with the neuronal and neuromuscular functions of these tissues. Their sensitivity to organophosphate inhibitors also highlights their usefulness as biomarkers of neurotoxic pesticide exposure.

Compared with *O. mossambicus*, *P. sarana* displayed a more complex esterase pattern, with five mobility zones. The presence of four bands in gill, liver, and intestine suggests higher biochemical diversity in tissues involved in environmental contact, digestion, and detoxification. ER esterases were prominent in gill and liver, indicating the presence of esterases that were not affected by the inhibitor conditions used. The Rm 0.86 intestinal band, which was unique to *P. sarana*, may represent a tissue-specific isozyme associated with intestinal metabolism or xenobiotic processing.

The widespread Rm 0.50 and 0.33 bands in *P. sarana* suggest conserved esterase fractions; however, their inhibitor classifications varied substantially among tissues. For example, the Rm 0.50 zone was identified as ChE in gill and intestine, CE in liver and eye, Esdp in brain, and CHsp in muscle. Likewise, the Rm 0.33 zone was classified as ChE, CHsp, CE, or ArE depending on tissue. These differences reinforce that electrophoretic mobility alone cannot establish enzyme identity. Future work should combine zymography with substrate-specific assays, protein quantification, and molecular approaches such as transcript analysis or mass spectrometry.

Overall, the two fishes exhibited distinct tissue-specific esterase signatures. *P. sarana* had a larger number of esterase zones and greater diversity of esterase classes than *O. mossambicus*. Such profiles may serve as baseline biochemical markers for species identification, physiological studies, and monitoring of pesticide or other xenobiotic exposure in freshwater ecosystems.

V. CONCLUSION

Esterase zymograms demonstrated distinct tissue- and species-specific patterns in *O. mossambicus* and *P. sarana*. *O. mossambicus* showed three esterase zones, whereas *P. sarana* showed five. Liver, gill, and intestine generally exhibited greater esterase diversity, consistent with their major roles in detoxification and environmental interaction. Cholinesterase-rich neural and sensory tissues may be particularly useful for evaluating organophosphate-related neurotoxicity. These findings provide baseline information

for future ecotoxicological, physiological, and molecular studies.

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