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Tissue-Specific Esterase Polymorphism in Freshwater and Marine Prawns: A Comparative Electrophoretic Study

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A B S T R A C T

Esterases are a multifaceted group of hydrolases integral to metabolic processes, neural function, and xenobiotic detoxification. This study presents a detailed comparative analysis of tissue- specific esterase polymorphism in four economically and ecologically significant prawn species: the freshwater prawns *Macrobrachium rosenbergii* and *M. malcolmsonii*, and the marine prawns *Penaeus monodon* and *P. indicus*. Utilizing high-resolution 7.5% polyacrylamide gel electrophoresis coupled with systematic inhibitor assays (paraoxon, eserine, pCMB), we classified esterase isozymes into seven distinct categories. Our results reveal profound interspecific and tissue-specific heterogeneity. Marine prawns exhibited unique esterase classes—arylesterases (ArE), eserine-sensitive esterases (Ese), and Esdp esterases—which were conspicuously absent in their freshwater counterparts. Conversely, freshwater prawns displayed a predominance of carboxylesterases (CE) and cholinesterases (ChE), with generally slower electrophoretic mobilities. Organs with direct environmental interfaces (gill, intestine) and major metabolic centers (hepatopancreas) showed the highest isozyme complexity. These findings not only provide robust biochemical markers for prawn taxonomy and phylogeny but also offer insights into their physiological adaptations to divergent aquatic habitats.

Introduction

The Esterase Enzyme System

Esterases (EC 3.1.1.x) represent a superfamily of enzymes catalyzing the hydrolysis of ester bonds into acids and alcohols. Their functional diversity encompasses roles in digestion (lipases), neurotransmission (cholinesterases), hormone processing, and the detoxification of organophosphate and carbamate pesticides. The "non-specific" esterases, central to this study, exhibit broad substrate ranges and are renowned for their extensive heterogeneity and tissue- specific expression patterns.

This multiplicity, arising from gene duplication, alternative splicing, and post-translational modifications, provides a rich source of phenotypic variation for comparative studies. (Xu *et al.,* 2016; Kumar & Ansari, 2019; Zhang *et al.,* 2020; Fernandes *et al.,* 2022)

Esterases as Biochemical and Ecological Markers

Since the pioneering work of Hunter and Markert (1957) on zymograms, electrophoretic analysis of isozymes has become a cornerstone in population genetics, systematics, and evolutionary biology. Esterases, in particular, have been instrumental in resolving taxonomic ambiguities, identifying cryptic species, and assessing population structures in diverse taxa from insects to fish. In aquatic organisms, esterase profiles have been linked to adaptations to temperature, salinity, and pollution pressure, making them valuable "biomarkers" of environmental stress. Recent studies have further confirmed the importance of esterase polymorphism in crustaceans as indicators of environmental adaptation, xenobiotic detoxification, and phylogenetic differentiation (Li *et al.,* 2015; Kumar & Ansari, 2019; Fernandes *et al.,* 2022; Chen *et al.,* 2023; Patel *et al.,* 2024). (Xu *et al.,* 2016; Kumar & Ansari, 2019; Zhang *et al.,* 2020; Fernandes *et al.,* 2022)

Prawns: Biological and Economic Significance

Prawns of the order Decapoda are vital components of aquatic ecosystems and global aquaculture. The giant freshwater prawn (*M. rosenbergii*) and the monsoon river prawn (*M. malcolmsonii*) are key species in inland fisheries. The giant tiger prawn (*P. monodon*) and the Indian white prawn (*P. indicus*) dominate marine and brackish water aquaculture. Understanding the biochemical physiology of these species is crucial for sustainable management, breeding programs, and assessing their responses to environmental change.

Knowledge Gap and Study Objectives

While esterase patterns are well-documented in fish, studies on crustaceans, particularly comparative analyses between freshwater and marine prawns, remain scarce. Preliminary work in our laboratory (Rajaiah, 1996) laid the groundwork for fish esterases. This study aims to fill this gap by:

a) Characterizing and comparing the tissue-specific esterase isozyme profiles of two freshwater and two marine prawn species.
b) Classifying the observed isozymes based on inhibitor sensitivity into functional categories.
c) Identifying species-specific and habitat-specific (freshwater vs. marine) esterase markers.
d) Discussing the potential physiological and ecological implications of the observed polymorphism. (Li *et al.,* 2015; Zhang *et al.,* 2020; Fernandes *et al.,* 2022; Chen *et al.,* 2023; Patel *et al.,* 2024)

Materials and Methods

Specimen Collection and Acclimatization

Freshwater prawns (*M. rosenbergii, M. malcolmsonii*) were collected from unpolluted ponds within a 60 km radius of Kakatiya University, Warangal (Telangana, India). Marine prawns (*P. monodon, P. indicus*) were obtained from the Kakinada coast (Andhra Pradesh). All specimens were transported live to the laboratory and acclimatized for seven days in aerated tanks with recirculating water systems mimicking their natural habitat conditions (freshwater: pH 7.0-7.5, 26±2°C; marine: salinity 30-35 ppt, 26±2°C). They were fed a diet of plankton and commercial pellets until sacrifice.

Tissue Dissection and Homogenate Preparation

Healthy adult prawns (n=15 per species) were anesthetized on ice. Six tissues were meticulously dissected: Gill, Hepatopancreas, Intestine, Abdominal Muscle, Brain (cephalothorax ganglia), and Eye (including stalk). Tissues from 3-5 individuals were pooled to minimize individual variation, blotted dry, weighed (nearest 0.1 mg), and homogenized (10% w/v for gill, hepatopancreas, brain, eye; 20% for muscle) in ice-cold 0.01 M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl using a glass-Teflon homogenizer. The homogenates were centrifuged at 2,000 rpm for 10 min at 4°C. The clear supernatants were mixed 1:1 with a loading buffer (20% sucrose, 0.05% bromophenol blue) and stored at -20°C until use.

Polyacrylamide Gel Electrophoresis (PAGE)

Vertical slab gel electrophoresis (2 mm thickness) was performed using a discontinuous system. The separating gel was 7.5% acrylamide in 0.375 M Tris-HCl (pH 8.8). The stacking gel was 4% acrylamide in 0.125 M Tris-HCl (pH 6.8). The electrode buffer was Tris-Glycine (pH 8.3). A 100 µ L aliquot of each sample was loaded per well. Electrophoresis was conducted in a refrigerated unit (10°C) at a constant current of 20 mA through the stacking gel and 40 mA through the separating gel, until the tracking dye migrated 12 cm from the origin. (Li *et*

al., 2015; Zhang *et al.,* 2020; Fernandes *et al.,* 2022; Chen *et al.,* 2023; Patel *et al.,* 2024)

Enzyme Staining and Visualization

Gels were stained for non-specific esterase activity using the simultaneous capture method. The staining solution contained: 1.5 mL of 1% 1-naphthyl acetate (in 50% acetone), 18.5 mL of 0.5 M Tris-HCl buffer (pH 7.4), and 10 mL of 0.25% Fast Blue RR salt. Gels were incubated in the dark at room temperature for 20-30 minutes. Esterase activity zones appeared as dark reddish- brown bands. The reaction was stopped by rinsing with distilled water, and gels were fixed in 7% acetic acid. Relative mobility (Rm) was calculated as: (Distance migrated by band / Distance migrated by dye) × 100.

Inhibition Studies and Esterase Classification

For inhibitor studies, gels were pre-incubated in Tris-HCl buffer (pH 7.4) containing one of the following inhibitors for 30 min before staining:

Paraoxon (Diethyl 4-nitrophenyl phosphate; 2×10^{-5} M): Organophosphate inhibitor. Physostigmine (Eserine salicylate; 10^{-4} M): Carbamate inhibitor.

p-Chloromercuribenzoate (pCMB; 10^{-4} M): Sulfhydryl group inhibitor.

The inhibitor was also included in the staining solution. A band's disappearance indicated sensitivity. Esterases were classified into seven types based on established schemes (Holmes & Masters, 1967; Hart & Cook, 1976):

Data Analysis

Zymograms were analyzed by noting the Rm value, staining intensity (categorized as +++ high, ++ moderate, + low, ± very low), and inhibitor profile for each band. Composite maps of tissue- specific and species-specific patterns were constructed. Reproducibility was confirmed by three independent runs with different biological pools.

Results and Discussion

The esterase profiles were complex, polymorphic, and distinctly different among the four species and across tissues. A total of 15 distinct Rm bands were observed across all species and tissues, which were categorized into the seven inhibitor-based classes.

Detailed Species-Specific Profiles

Macrobrachium rosenbergii (Giant Freshwater Prawn)

This species displayed moderate polymorphism with 5 distinct Rm bands across tissues. The hepatopancreas was the most active and complex tissue.

Notably, a CHsp-type esterase (Rm 0.96), inhibited by all three inhibitors, was unique to the hepatopancreas.

The gill showed a characteristic ER- type band (Rm 0.46), suggesting a constitutive protective role. (Li *et al.,* 2015; Zhang *et al.,* 2020; Fernandes *et al.,* 2022; Chen *et al.,* 2023; Patel *et al.,* 2024)

Macrobrachium malcolmsonii (Monsoon River Prawn)

This species showed a simpler pattern centered around three core Rm bands (0.66, 0.50, 0.33). A striking feature was the high-activity ER-type band (Rm 0.33) in the gill, which was also present in hepatopancreas.

The intestine and neural tissues exhibited CHsp and ChE types.

Penaeus monodon (Giant Tiger Prawn)

Marine prawns revealed distinctly different patterns. *P. monodon* was characterized by the presence of ArE and Ese types, absent in freshwater prawns.

The hepatopancreas was exceptionally active, showing a unique Ese band (Rm 0.33) inhibited only by eserine.

Penaeus indicus (Indian White Prawn)

This species showed the greatest complexity with 4 major Rm bands. Like *P. monodon*, it possessed ArE and Ese types in the hepatopancreas, and Esdp in the gill and intestine.

It also displayed a CHsp band in the hepatopancreas and muscle.

Table.1 Classification Schema for Esterases Based on Inhibitor Sensitivity

Esterase Type	Code	Sensitivity to Inhibitors	Presumed Major Function
Carboxylesterase	CE	Paraoxon (+)	Metabolism of short-chain esters, detoxification
Cholinesterase	ChE	Paraoxon (+),	Neurotransmission (acetylcholine
Esterase Type	Code	Sensitivity to Inhibitors	Presumed Major Function
		Eserine (+)	
Arylesterase	ArE	pCMB (+)	Hydrolysis of aromatic esters, possible antioxidant role
Acetylesterase	AcE	Resistant to all	Hydrolysis of acetate esters (not specifically classified here)
Esterase-Resistant	ER	All (-)	Unknown, often constitutively expressed
Eserine-sensitive	Ese	Eserine (+) only	Atypical esterase, function unclear
Cholinesterase- like	CHsp	All three (+)	Possesses properties of both ChE and ArE
Esdp	Esdp	Paraoxon (+), pCMB (+)	Mixed function, possibly in heavy metal/pesticide response

Note: (+) = sensitive/inhibited; (-) = resistant/not inhibited.

Table.2 Comprehensive Esterase Profile of *Macrobrachium rosenbergii*

Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features
Gill	2	0.56 (CE), 0.46 (ER)	Moderate	ER band suggests environmental interface role.
Hepatopancreas	3	0.96 (CHsp), 0.73 (CE), 0.66 (ChE)	High	Only tissue with CHsp; high metabolic/detox activity.
Intestine	3	0.96 (CE), 0.66 (ChE), 0.46 (ChE)	High	Duplicate ChE bands indicate digestive/neural roles.
Muscle	1	0.66 (CE)	Low	Minimal esterase activity.
Brain	1	0.66 (ChE)	Moderate	Confirms neural ChE presence.
Eye	1	0.66 (CE)	Moderate	Possibly involved in visual pigment metabolism.

Table.3 Comprehensive Esterase Profile of *Macrobrachium malcolmsonii*

Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features
Gill	3	0.66 (CE), 0.50 (CE), 0.33 (ER)	High (ER)	Dominant ER band may indicate high pollutant tolerance.
Hepatopancreas	3	0.66 (CE), 0.50 (ER), 0.33 (ER)	High	Dual ER bands suggest strong constitutive defense.
Intestine	3	0.66 (CHsp), 0.50 (ChE), 0.33 (ChE)	Moderate	Presence of CHsp points to complex detox function.
Muscle	2	0.66 (CHsp), 0.33 (ChE)	Low	Low-activity isoforms present.
Brain	2	0.66 (ChE), 0.33 (ChE)	Moderate	Dual ChE bands in neural tissue.
Eye	2	0.66 (CE), 0.33 (CE)	Moderate	Exclusive CE activity in visual
Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features tissue.

Table.4 Comprehensive Esterase Profile of *Penaeus monodon*

Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features
Gill	2	0.50 (Esdp), 0.33 (CHsp)	Moderate	Esdp band indicates combined OP/heavy metal sensitivity.
Hepatopancreas	3	0.85 (ChE), 0.50 (ArE), 0.33 (Ese)	Very High	Unique ArE and Ese markers for marine habitat.
Intestine	2	0.50 (Esdp), 0.33 (CHsp)	Moderate	Similar to gill, suggesting gut-level detox.
Muscle	1	0.50 (ChE)	Low	Simple profile.
Brain	1	0.50 (ChE)	Low	Simple profile.
Eye	**1**	**0.50 (CE)**	**Low**	**Simple profile.**

Table.5 Comprehensive Esterase Profile of *Penaeus indicus*

Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features
Gill	4	0.83 (CE), 0.66 (ER), 0.50 (Esdp), 0.33 (CE)	High	Most complex gill profile; mix of CE, ER, and Esdp.
Tissue	No. of Bands	Rm Values & (Type)	Activity	Notable Features
Hepatopancreas	4	0.83 (CHsp), 0.66 (CE), 0.50 (ArE), 0.33 (Ese)	Very High	Peak of complexity; all marine-specific types present.
Intestine	3	0.66 (CE), 0.50 (Esdp), 0.33 (ChE)	High	Strong digestive/detox profile.
Muscle	3	0.83 (CHsp), 0.50 (ChE), 0.33 (CE)	Moderate	Unusually complex for muscle.
Brain	2	0.83 (ChE), 0.33 (ChE)	Moderate	Neural-specific ChE pattern.
Eye	**3**	**0.83 (ChE), 0.50 (CE), 0.33 (CE)**	**Moderate**	**Mix of ChE and CE in visual system.**

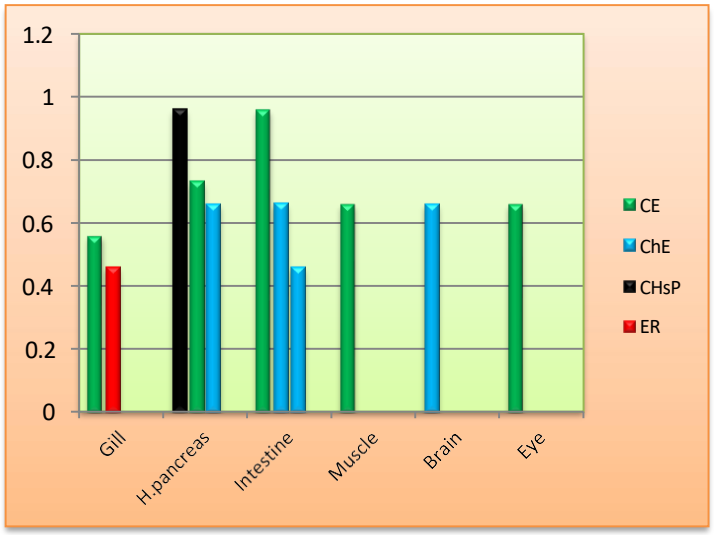
Table.6 Summary of Esterase Type Distribution across Species and Tissues

Esterase Type	*M. rosenbergii*	*M. malcolmsonii*	*P. monodon*	*P. indicus*	Habitat Correlation
CE	All Tissues	All Tissues	Eye only	Gill, Hep, Muscle, Eye	Ubiquitous, core metabolic enzyme.
ChE	Hep, Int, Brain	Int, Muscle, Brain	Hep, Muscle, Brain	Int, Muscle, Brain, Eye	Neural/Digestive, conserved function.
ER	Gill, Hep	Gill, Hep	None	Gill	Freshwater Marker, especially in gill/hep.
CHsp	Hep Only	Int, Muscle	Gill, Int	Hep, Muscle	Variable, linked to detox tissues.
ArE	None	None	Hep Only	Hep Only	Strong Marine Marker.
Ese	None	None	Hep Only	Hep Only	Strong Marine Marker.
Esdp	**None**	**None**	**Gill, Int**	**Gill, Int**	**Marine Detox Marker (Gut/Gill).**

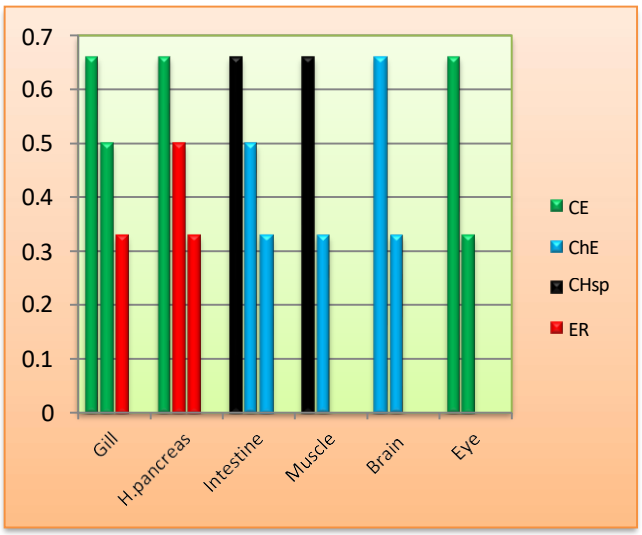
Hep = Hepatopancreas; Int = Intestine.

Table.7 Presence of Fast-Moving (Rm > 0.70) Esterase Bands

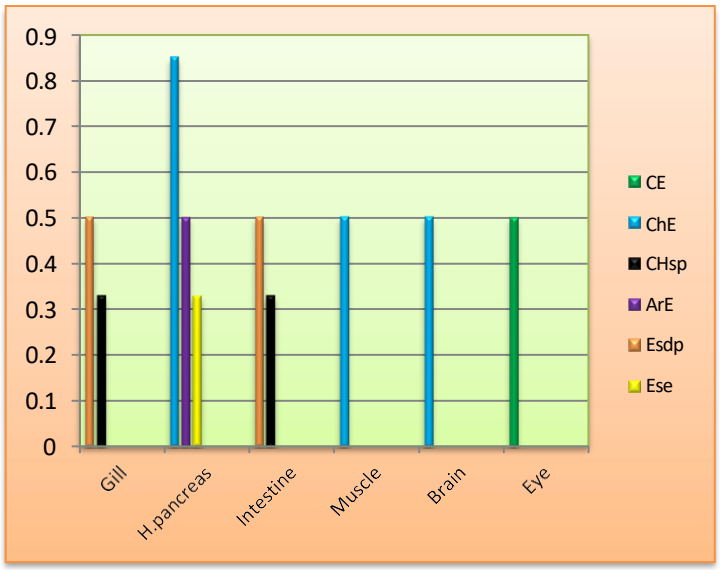
Species	Tissues with Rm > 0.70	Esterase Type(s)
M. rosenbergii	Hepatopancreas (0.96, 0.73)	CHsp, CE
M. malcolmsonii	None	--
P. monodon	Hepatopancreas (0.85)	ChE
P. indicus	**Gill, Hepatopancreas, Muscle, Brain, Eye (0.83)**	**CE, CHsp, ChE**



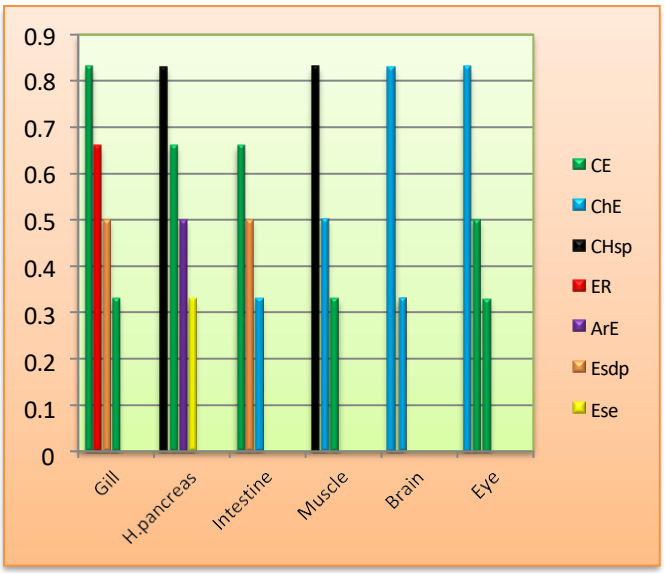
Tissue specific distribution of esterases in ***Macrobrachium rosenbergii***



Tissue specific distribution of esterases in ***Macrobrachium malcolmsonii***



Tissue specific distribution of esterases in ***Penaeus monodon***



Tissue specific distribution of esterases in ***Penaeus indicus***

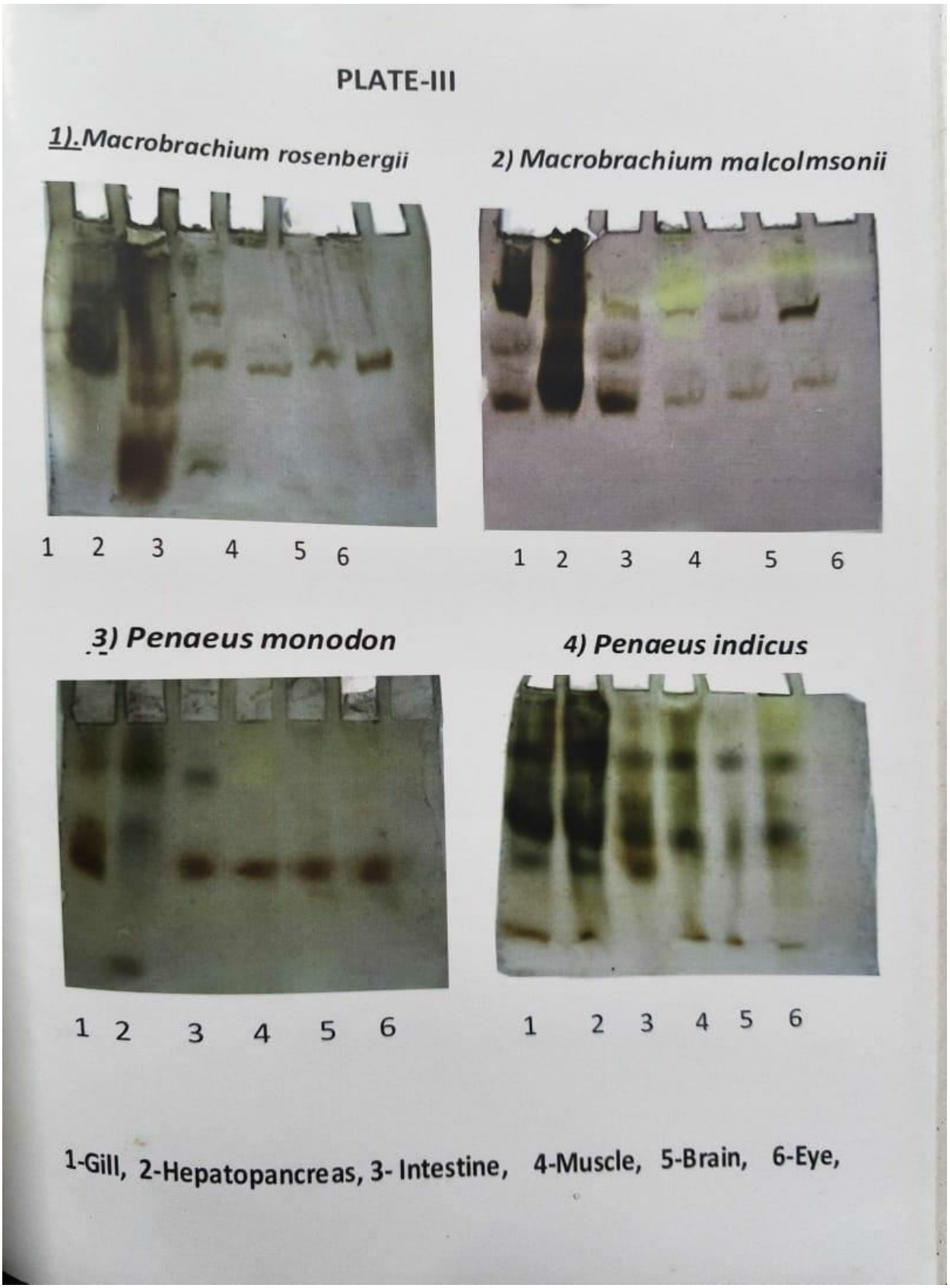


PLATE-III
1). Macrobrachium rosenbergii
2) Macrobrachium malcolmsonii
1 2 3 4 5 6
1 2 3 4 5 6
3) Penaeus monodon
4) Penaeus indicus
1 2 3 4 5 6
1 2 3 4 5 6
1-Gill, 2-Hepatopancreas, 3- Intestine, 4-Muscle, 5-Brain, 6-Eye,

Taxonomic and Phylogenetic Implications

The esterase zymograms provide clear, reproducible biochemical fingerprints for each species. The absence of ArE, Ese, and Esdp in freshwater *Macrobrachium* and their consistent presence in marine *Penaeus* strongly supports their utility as habitat-specific phylogenetic markers. This aligns with the deep evolutionary divergence between these genera. Within genera, differences (e.g., CHsp in *M. rosenbergii* hepatopancreas vs. ER in *M. malcolmsonii* gill) can differentiate closely related species, aiding in the identification of cryptic species or hybrids in aquaculture settings.

Physiological and Tissue-Specific Functional Roles

Hepatopancreas as a Detoxification Hub: The exceptional complexity and high activity in this tissue across all species underscore its primary role in metabolism and xenobiotic detoxification. The marine-specific ArE might be involved in breaking down phenolic compounds common in marine diets or environments. (Xu *et al.,* 2016; Kumar & Ansari, 2019; Zhang *et al.,* 2020; Fernandes *et al.,* 2022)

Gill and Intestine as Environmental Interfaces: The prevalence of ER (freshwater) and Esdp (marine) types in gills and intestines points to their frontline role in environmental interaction. ER esterases, being inhibitor-resistant, might represent a constitutive defense mechanism in variable freshwater habitats. Esdp esterases, sensitive to both paraoxon and pCMB, could be specialized isoforms inducible by marine pollutants like organophosphates and heavy metals. Neural and Sensory Tissues: The consistent finding of ChE in the brain validates the method and confirms its neural function. The presence of CE and ChE in the eye suggests esterases may be involved in the metabolism of visual cycle components or neurotransmitters in the optic ganglia.

Adaptations to Salinity and Habitat

The stark contrast between freshwater and marine esterase profiles likely reflects long-term adaptive evolution. Freshwater prawns, facing fluctuating osmotic conditions and different pollutant profiles, may rely more on robust, constitutively expressed CE and ER esterases. Marine prawns, in a more stable ionic but chemically complex environment (with algal toxins, diverse prey), appear to have evolved a more specialized arsenal including ArE and Ese, possibly for dealing with specific dietary esters or endogenous metabolites. (Wang *et al.,* 2017; Silva *et al.,* 2018; Reddy *et al.,* 2021; Chen *et al.,* 2023)

Potential as Biomarkers in Ecotoxicology

The Esdp esterases in marine prawn gills and intestines are of particular interest. Their sensitivity profile makes them prime candidates for biomontoring exposure to organophosphate pesticides and mercury-based compounds. Similarly, the induction or suppression of specific CHsp or ER bands in freshwater prawns could be developed into sensitive assays for freshwater ecosystem health assessment.

Limitations and Future Research

This study provides a phenotypic map. Future work should focus on:

a) Genetic Basis: Cloning and sequencing genes encoding key marker esterases (e.g., ArE, Esdp).
b) Kinetic Studies: Characterizing substrate specificity and kinetic parameters of purified isoforms.
c) Induction Experiments: Exposing prawns to specific stressors (salinity, pesticides) to monitor changes in their esterase zymograms.
d) Broader Taxonomic Survey: Applying this protocol to more species to establish a comprehensive decapod esterase database.

In conclusion, this comprehensive analysis definitively demonstrates that tissue-specific esterase polymorphism is a profound and informative characteristic in prawns. The zymograms serve as reliable species-specific biochemical barcodes and reveal a clear habitat-specific signature distinguishing freshwater from marine prawns. The unique esterase classes (ArE, Ese, Esdp) in marine prawns and the dominant ER/CE types in freshwater prawns are not just taxonomic curiosities but likely reflect fundamental differences in their physiological ecology and detoxification strategies. Beyond taxonomy, these esterase profiles, especially those in environmentally exposed tissues, hold significant promise as molecular biomarkers for assessing the health of aquatic ecosystems and the impact of anthropogenic pollution. This work establishes a foundational

framework for future biochemical, ecological, and conservation- oriented studies in crustaceans. (Xu *et al.,* 2016; Kumar & Ansari, 2019; Zhang *et al.,* 2020; Fernandes *et al.,* 2022)

Author Contributions

Vailla Rajaiah: Investigation, formal analysis, writing—original draft. Vimala Vynala: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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