DOI: https://doi.org/10.36094/sc.v91.2025.Effect_of_Ecosystems_on_Gut_Enzymes.Chakraborty.187

ARTICLE

*Sci. and Cult.* 91 (3–4) : 187-190 (2025)

# EFFECT OF ECOSYSTEMS ON GUT ENZYMES IN AGE AND SEX-DEPENDENT OF ANADROMOUS HILSA, *TENUALOSA ILISHA* (HAM, 1822)

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*This study investigated age- and sex-dependent variations in digestive enzymes of migratory Hilsa (Tenualosa ilisha) across freshwater, brackish, and marine habitats. Results showed significant sex-based variations in amylase and protease ($p < 0.05$), while lipase showed significant differences ($F = 4.745$, $p < 0.05$). Protease activity was significantly influenced by the ecosystem ($F = 109.6$, $p < 0.001$). Enzyme responses varied by habitat with dietary adaptations in different salinities. The observations led to optimizing the Hilsa feed based on age, sex, and habitat.*

***Keywords:*** *Habitat, Salinity, Feeding Habit, Correlation, Hilsa, Migration*

## *Introduction*

In South Asia, the Hilsa (*Tenualosa ilisha*) is a highly valued migratory fish, known for its cultural and economic significance[1]. It is an euryhaline and anadromous species, meaning it can tolerate a wide range of salinities and primarily resides in marine waters but migrates to freshwater for spawning[2]. Various factors during migration affect the Hilsa, but the fish is capable of adapting well to environmental changes. One such adaptation involves maintaining osmotic pressure, which influences energy allocation and may divert resources toward the production of digestive enzymes, potentially affecting digestion efficiency throughout the migration process. Hilsa's digestive enzymes have evolved to efficiently break down diverse foods across various salinities in marine, brackish, and freshwater habitats. Additionally, the fish can adjust its enzyme synthesis in estuarine environments, where salinity fluctuates frequently. Hilsa prefers brackish water regions due to subsurface oxygen, relatively low salinity, rapid tidal movement, high turbidity, heavy siltation, and abundant plankton development[3]. For example, higher salinity can enhance the activity of specific digestive enzymes, aiding in the absorption of nutrients from the diverse diet available in these environments. Amylase and protease are essential enzymes in cellular metabolism for microbes, plants, and animals. These enzymes accelerate the breakdown of macromolecules into smaller components, which are then used by cells to create new biomolecules or generate energy. Amylases hydrolyze starch, glycogen, and other polysaccharides into short-chain sugars, playing a crucial metabolic role. Proteases break down proteins into small peptides and amino acids and are found in all living organisms. These enzymes are classified into two families—alkaline and acid proteases—based on the optimal pH for their activity. The present study aims to investigate the activity of key digestive enzymes, such as amylase, lipase,

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and protease, secreted from the pancreas into the small intestine of Hilsa, and their relationship with different salinity levels.

## Materials and Methods

***Sample Collection, Preservation, Processing:*** A total number of 90 Hilsa (male and female) were collected from three different saline environments -freshwater- Farakka (24p 48'33.44" N; 87p 55'8.37" E), brackish water- Frasergunj (21p 34'44.76" N; 88p 14'59.72" E) and marine water- Gangasagar (21p 25'13.13" N; 88p 5'8.06" E) (Fig. 1). 30 Hilsa representing 15 male and 15 female from each ecosystem. Gut samples were taken from the fish after dissected and placed in a 0.25M sucrose solution. While being transported from the sample site to the laboratory, the samples were kept in dry ice. After homogenizing the materials with the QAIGEN tissuelyser II (QAIGEN, Hilden, Germany), then centrifuged for ten minutes at 10,000 rpm. Before enzymatic analysis, the supernatant was then collected in sterile 2 mL Eppendorf tubes and kept at -40°C.

***Gut Enzymes Analysis:*** To determine the amylase concentration 3,5-dinirtosalicylic acid (DNS) was used followed by previous author method[4]. Using the method developed by authors[5] in 1932, lipase activity was assessed. The casein digestion method was used to evaluate protease activity[6].

***Statistical Analysis:*** In this study, one-way analysis of variance technique (ANOVA) followed by post hoc Tukey's test was conducted to check the significant differences among enzymes. PCA was conducted to examine the relations among the enzymes across different saline ecosystems and between males and females using the Factominer package[7]. A box plot was generated using ggplot2[8]. Spearman rank correlation was conducted to examine the associations among enzymes, ecosystems and sex. The statistical analysis in this study was visualized by using the R software version 4.4.2 (core team, 2024). Data are shown as means ± standard deviation (S.D.). Differences were considered statistically significant at $p<0.05$.

## Results and Discussion

It is well established that many enzymatic activities decrease with age in animals[9-13]. Several comparative studies on digestive enzymes in different fish species have been conducted[14- 19]. While it is known that fish digestive enzymes are qualitatively similar to those found in other vertebrates, the digestive processes of fish are less understood compared to mammals. This study aims to evaluate the salinity and optimal enzyme activity of *Tenualosa ilisha* (Indian shad) across different saline ecosystem.

A one-way analysis of variance (ANOVA) was used to assess the effects of sex and salinity on enzyme levels in Hilsa collected from freshwater, brackish, and marine environments. The results showed that amylase and protease levels did not differ significantly between sexes ($p > 0.05$). However, lipase activity displayed a significant difference between sexes ($F = 4.745$, $p < 0.05$) (Figure 2). Regarding varied ecosystems based on salinity, no significant difference in amylase and lipase activity was found across the ecosystems ($p > 0.05$), but protease activity varied significantly with salinity ($F = 109.6$, $p < 0.001$). Tukey's post hoc test indicated no significant differences in enzyme activity between groups with the same letters (i.e., 'a', 'a') ($p > 0.05$). The descriptive statistics of the enzymes across the ecosystems are represented in Table.1.

**Table 1. Descriptive statistics for enzyme activities in *Tenualosa ilisha* sampled across three salinity environments**

| Enzyme | Ecosystem | Mean ± SD | Minimum | Maximum |
|---|---|---|---|---|
| Amylase | FW | 6.03 ± 3.74$^{a}$ | 1.29 | 24.53 |
| | BW | 4.47 ± 3.67$^{a}$ | 0.36 | 15.26 |
| | MW | 4.62 ± 3.06$^{a}$ | 1.22 | 10.22 |
| Protease | FW | 2.28 ± 1.71$^{c}$ | 0.07 | 13.07 |
| | BW | 11.41 ±3.08$^{a}$ | 4.99 | 16.17 |
| | MW | 5.82 ± 4.12$^{b}$ | 0.99 | 12.54 |
| Lipase | FW | 11.83 ±4.63$^{a}$ | 4.47 | 26.09 |
| | BW | 14.01 ±2.43$^{a}$ | 9.4 | 17.83 |
| | MW | 12.13 ±4.05$^{a}$ | 6.78 | 21.36 |

Values with the same letters (i.e., 'a', 'a') indicate no significant difference in enzyme activity between groups as determined by Tukey's post hoc test ($p > 0.05$).

***Principal Component Analysis (PCA):*** Principal Component Analysis (PCA) was conducted to explore the data structure and relationships among amylase, protease, and lipase enzymes, and their associations with different ecosystems and sexes (Fig. 3). Despite significant correlations among variables, the low Kaiser-Meyer-Olkin (KMO = 0.49) value suggested limited suitability for factor analysis. PCA revealed that axes 1 and 2 explained a substantial portion of the variance: 34.71% and 27.12%, respectively, for a cumulative variance of 61.82%. Axis 1 was primarily characterized by high positive loading for

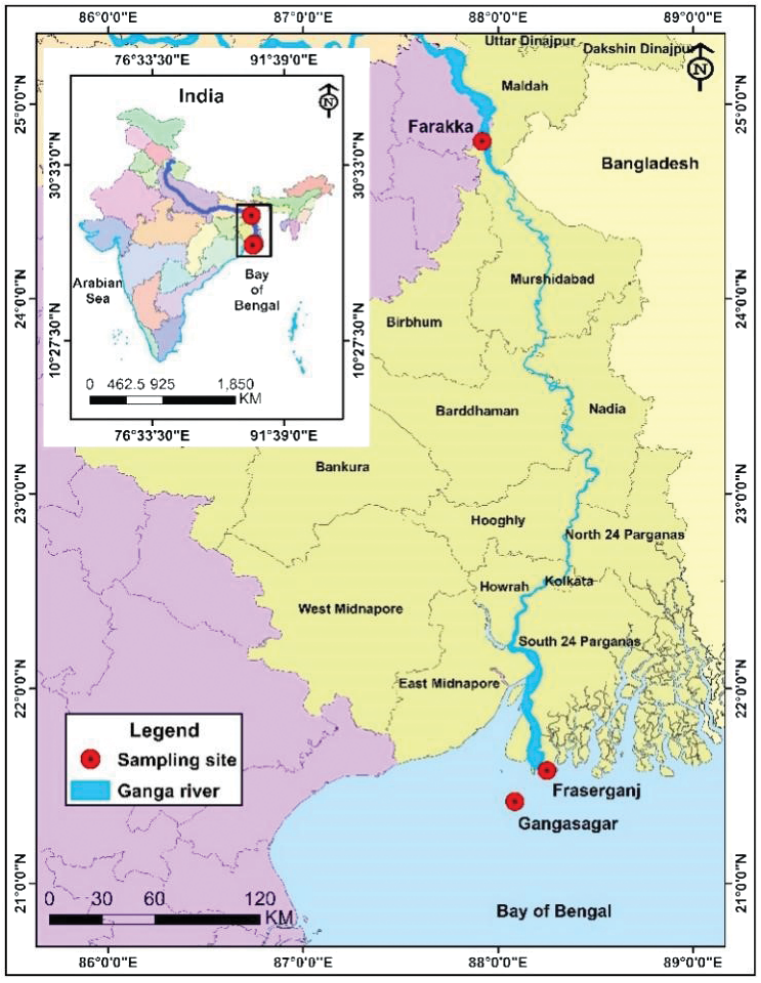


**Fig. 1.** Hilsa sample collection sites

amylase and strong negative loading for salinity. Protease and lipase were also negatively correlated with salinity. Axis 2 was dominated by a strong negative loading for lipase and a moderate negative loading for amylase. This axis was positively associated with weight, indicating that higher PC2 scores corresponded to higher weight values, and negatively correlated with lipase and amylase, suggesting an inverse relationship with these enzymes.

Spearman's rank correlation analysis revealed a statistically significant positive correlation between amylase and in the low salinity ecosystem, freshwater (FW) (r = 0.22, p = 0.05). Conversely, protease (r = -0.33, p = 0.001) and lipase (r = -0.23, p = 0.05) exhibited significant negative correlations with the FW (Figure 4). For the moderate salinity, brackish water (BW), protease showed a significant positive correlation (r = 0.62, p = 0.001), while lipase exhibited a significant negative correlation (r = -0.29, p = 0.01). Regarding sex differences, lipase showed a significantly negative correlation with female groups (r = -0.21, p < 0.05) and a positive correlation with male groups (r = 0.21, p < 0.05). Weight also significantly influenced enzyme activity, with amylase (r = 0.27, p < 0.01) and protease (r = 0.20, p < 0.05) exhibiting positive correlations with weight.

The digestive enzyme activities in Hilsa suggest adaptations to dietary changes in different saline ecosystems. Euryhaline fish, such as Hilsa, undergo metabolic restructuring to meet the energetic demands of osmoregulation when acclimating to varying salinities [20, 21]. In particular, the protease activity in young Hilsa was higher, with the marine environment showing greater activity than the freshwater environment. Amylase and lipase activities reflect the fish's ability to effectively utilize fats and carbohydrates in different environments [22]. Studies of these enzymes can assist in addressing nutritional issues, such as

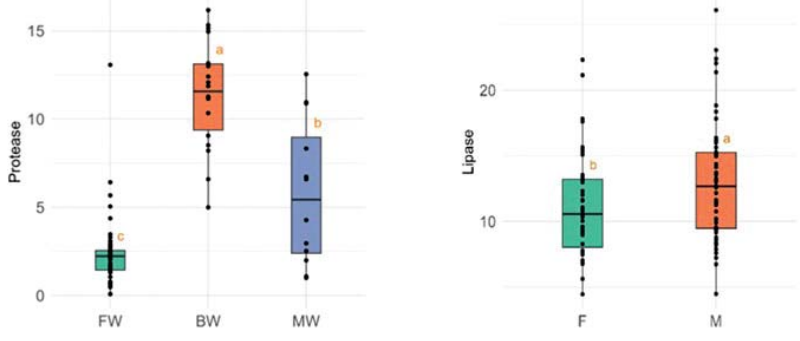


**Fig. 2.** Enzymes showing significant levels across salinity gradients and sex in *Tenualosa ilisha*

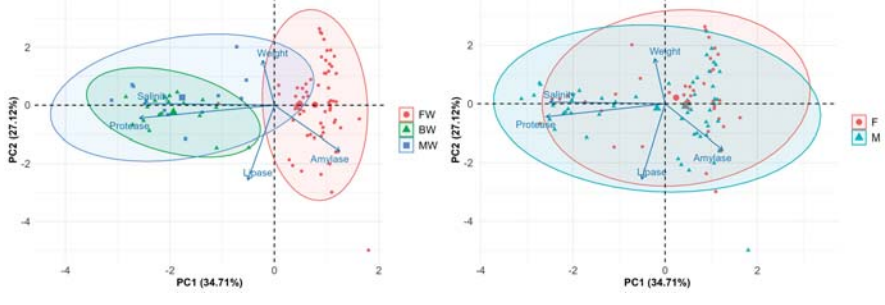


**Fig. 3.** PCA analysis of Hilsa different gut enzymes across varied ecosystems and sexes

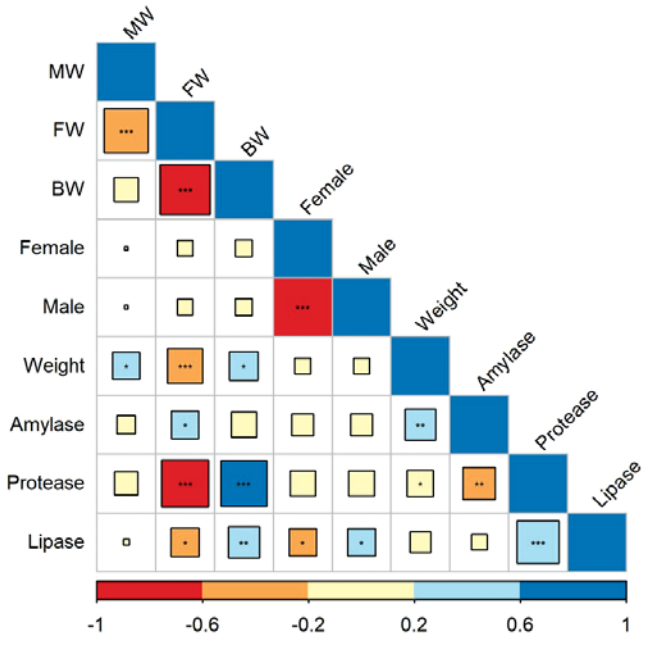


**Fig. 4.** Correlation plot of *T. ilisha* between ecosystems, sex and enzymes

formulating appropriate artificial feeds and understanding the fish's nutritional physiology[23,24]. Research on Hilsa feeding behavior throughout its life span shows that juveniles primarily feed on zooplankton for the first five to six months in freshwater. As they mature, their feeding habits shift towards phytoplankton, with seasonal fluctuations influencing these dietary preferences[25].

## Conclusion

Research on the digestive system enzymes of *Tenualosa ilisha* is crucial for effective Hilsa feed development. Understanding the species' habitat conditions and dietary habits is essential before introducing it from the wild into captivity. This study provides preliminary insights into the dynamics of gastrointestinal enzymes in Hilsa interacting with age, sex and varied ecosystems, particularly on salinity. Such information is valuable for managing feeding trials in captivity, ultimately contributing to more effective conservation efforts.

## Acknowledgments

The authors are grateful to National Mission for Clean Ganga for providing us the platform to perform the experiments and carry out the research activities and also to ICAR- Central Inland Fisheries Research Institute for providing us with all the research support and facilities. ❐

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