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# Studies on the feeding habit and digestive enzyme activities in three small indigenous fish species from Assam, India

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Relative gut length

Gastro-somatic index

Digestive enzyme

**ABSTRACT**

Knowledge of the feeding habit and the digestive physiology of a fish is important in making appropriate strategies for feed development and successful culture. Nutrient-rich small indigenous fish species (SIFs) are abundant in Assam, India. *Puntius sophore*, *Mystus tengara*, and *Trichogaster fasciata* of Gossaigaon, Assam are important SIFs for the local rural population, and also potential candidates for ornamental fish culture. The present study aims to evaluate the feeding habit and digestive enzyme activities of these species. Data obtained from the relative gut length and gut content analysis suggested that *M. tengara* is a carnivorous fish and the rest two fishes are omnivorous in habit. Further, the relative gut length was highest in *T. fasciata* (4.20±0.45) and lowest in *M. tengara* (0.55±0.11). Digestive enzyme activity indicates a correlation with the dietary habit of the fish. Further, total protease, trypsin, and amylase activity was reported highest in *P. sophore*. Acid protease pepsin was found to be significantly higher in *M. tengara* complementing its carnivorous habit and gut anatomy. The present study has established some important information on the digestive enzyme characteristics and feeding habits of the three fish species. This information might be useful in the development of suitable feed for the fish species for their culture.

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## 1 Introduction

Small indigenous fishes are an important group of fish with immense potential for culture both as food and ornamental fish. These are usually small with a maximum length of 25 cm to 30 cm and is considered a cheap source of proteins, vitamins, and minerals, especially for the rural population (Bhutia et al. 2021). In India maximum diversity of SIFs has been recorded from the northeast region (Duarah and Das 2019). SIFs are a high source of macro and micronutrients which are vital for human nutrition. Considered 'weed fishes', this category of fish has not been explored enough for mass culture and propagation. Although the majority (90%) of the population in Assam, India consumes fish, the increased fish production is yet to meet the growing population's demand (Yadav et al. 2020). Local indigenous fish species are good candidates for aquaculture species diversification and expansion as they are nutrient-rich and readily accepted by the people. But their culture is not very popular because of a poor understanding of their biology especially feeding habits and digestive physiology. Proper knowledge about the food and feeding habits of fish is essential for understanding the nutritional requirement of the species (Emmanuel et al. 2019) required for the successful development of an artificial culture system (Khan et al. 2022), and for the production and exploitation of the fish stocks (Meshram et al. 2022). The true natural feeding habit of a fish species can be better understood by in vivo studies (Khabade 2015). Information on the food and feeding pattern of a species is important to understand its nutritional requirement, distribution, and interaction with other organisms, and also for proper management (Gogoi et al. 2020; Kumar et al. 2022). Generally, fish are known to have high flexibility in digestive processes, and it depends on many factors. Digestive enzymes and their relationship with the composition of ingested food are essential to comprehending the feeding biology of a fish species (Almeida et al. 2018) and developing appropriate nutritional strategies.

*Puntius sophore* (Cypriniformes, Cyprinidae), *Mystus tengara* (Siluriformes, Bagridae), and *Trichogaster fasciata* (Anabantiformes, Osphronemidae) are freshwater fish species widely distributed throughout the Indian subcontinent in habiting small ponds, wetlands, lakes, and slow-flowing streams and rivers (Rahman et al. 2019; Mitu et al. 2019; Kumar et al. 2021). These species are important food fish, especially for the rural poor people. *M. tengara* is known to have good taste and a high nutrient profile. It is also reported to have good protein content (Ahmed et al. 2012) and is an indigenous ornamental fish with good export value (Gupta and Banerjee 2014). *T. fasciata* is an important species for small fish catchers with high commercial values both as ornamental and food fish (Rahman et al. 2019)

Food and feeding habits have been reported for several fish species (Alam et al. 2020; Dutta et al. 2020; Jewel et al. 2020; Kumar et al. 2022; Velasco-Reyes et al. 2022). But very few works have been reported on fishes from freshwater bodies in Assam, India especially in lower Assam (Gogoi et al. 2020). There is a scarcity of work on the digestive enzyme activity and its correlation with the feeding habit of SIFs of the region. The present investigation aimed to study the feeding habit and digestive enzyme activity of three important SIFs, *P. sophore*, *M. tengara*, and *T. fasciata*, found in the natural water bodies of the Gossaigaon, Assam, India.

## 2 Materials and Methods

### 2.1 Collection of fish

Adults of three small indigenous fish species (40 individuals for each species) from different families, *P. sophore* (length: 7.40±1.15 cm, weight: 2.84±1.25 g), *M. tengara* (Length: 9.23±0.84 cm, weight: 2.95±0.66 g) and *T. fasciata* (Length:8.69±0.64 cm, weight: 5.33±1.06 g) were collected randomly from the different natural water bodies and landing sites of the local river, Haraputa, in the Gossaigaon, Kokrajhar district of lower Assam (26°26'42.7" N, 89°56'39.4" E). The fish were identified with the help of standard keys and literature (Talwar and Jhingran 1991; Vishwanath et al. 2007; Froese and Pauly 2021). For digestive enzyme study, live fish samples were collected and transported to the laboratory facility at the Department of Zoology, Bodoland University, Assam, India. Representatives of each species were stored separately in 10% formalin to preserve their morphological structure for identification.

### 2.2 Relative gut length (RGL) and Gastro-somatic index (Ga.SI)

The individual fish samples were washed and digestive tracts were dissected on an ice-cold platform. The length and weight of the dissected gastrointestinal tract were recorded. The characterization of different fish as carnivores, herbivores, and omnivores was done by using the method of RGL as a morphological variable while the feeding intensity was done by calculating the Ga.SI. The RGL (Al-Hussani 1949) and the Ga.SI (Bhatnagar and Karamchandani 1970) were calculated by using the following formulae.

$$\text{RGL} = \text{Total length of gut}/\text{Total length of fish}$$

$$\text{Ga.SI.} = (\text{Weight of gut} \times 100)/\text{Weight of fish}$$

### 2.3 Gut content analysis

For gut content analysis, the gut (preserved in 5% formalin to prevent further breakdown of food particles) of individual fish was dissected. The gut contents of each fish were then carefully emptied into a petri dish and observed under a compound microscope. The contents were analyzed qualitatively for identification into groups of plant or animal-derived food. These

were further identified into respective major groups like macrophytic plant parts, algae, diatoms, phytoplankton, zooplanktons, rotifers, cladocerans, and crustaceans, insects and their larvae, molluscs, small fish, detritus, and some as unidentified organic or inorganic matter. Most of the food contents were partially or significantly digested and hence identification up to species was not possible. Therefore, they were grouped under this broad classification by observing specific visible characteristics of each group.

**2.4 Preparation of crude enzyme extract**

Ten fish of each species were anesthetized with MS-222 (Tricaine methanesulfonate) and dissected on a cold platform maintained at 0-4°C. The digestive tracts were separated, cleaned, and weighed. The dissected digestive tract of each species of fish was homogenized in cold distilled water (1:10 w/v, tissue: water) and centrifuged (Eppendorf 5425R, Germany) at 10,000 g for 30 min at 4°C. The supernatant was separated, labeled, and used for the estimation of digestive enzyme activity.

**2.5 Digestive enzyme Activity**

Amylase activity was assayed by following Bernfeld's (1955) method. Briefly, starch solution (1% w/v) was used as a substrate, and it was incubated with the crude enzyme extract, 1% starch in phosphate buffer (0.1 M, pH 7.0), and NaCl for 1 hour at 37°C. The reaction was stopped by adding 3,5- DNSA (3,5-dinitro salicylic acid) and the absorbance was measured at 540nm using UV-Visible Spectrophotometer (Shimadzu 1900i, Japan). Specific amylase activity was expressed as milligram of maltose liberated per milligram protein in reaction mixture per hour at 37°C.

Trypsin activity was measured following the method of Erlanger et al. (1961). N*α*-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA, SRL, Mumbai India) was taken as substrate and incubated with the crude extract. The change in absorbance of the reaction mixture was recorded after 15 mins at 410nm in a UV-visible Spectrophotometer (Shimadzu 1900i, Japan). Specific trypsin activity was expressed as units per milligram protein per minute using the following formula:

$$\text{Activity units} = \frac{(\Delta\text{Abs } 410 \text{ nm/min} \times 1000 \times \text{mL of reaction mixture})}{(8800 \times \text{mg protein in reaction mixture})}$$

Pepsin activity was measured according to the method described by Anson (1938) using hemoglobin as the substrate. The activity was assayed using 500µl of 2% hemoglobin as a substrate. The reaction was started by adding 100 µl of crude extract and incubating at 37°C for 10 minutes. The reaction was stopped by adding 1000 µl Tricholoacetic acid (TCA, 5% w/v). The reaction mixture was centrifuged (12,000rpm, 5 min at room temperature) to separate the supernatant and its absorbance was measured at 280nm. Specific pepsin activity was calculated using the formula: Activity = Abs (test-blank)×1000/mg protein/min. The activity was expressed as Units/mg protein/min.

Total protease activity was measured following the method described by Garcia-Carreno (1992) and azocasein was taken as the substrate. Tris-HCl (SRL, India) was used as a buffer (pH 7.5). The reaction mixture consisting of crude extract, buffer, and azocasein was incubated at 25°C for 15 minutes, and thereafter, the reaction was stopped by adding 20% TCA. The samples were then centrifuged (10,000 rpm, 5 min at room temperature) to obtain the supernatant, and its absorbance was recorded at 366nm. The specific total protease activity was expressed as Abs (test-control)/mg protein in reaction mixture/min.

**2.6 Protein estimation**

Total soluble protein was measured according to Lowry et al. (1951). Bovine serum albumin (BSA) was used as the standard against the sample protein (1mg/ml).

**2.7 Statistical Analysis**

Data values are represented as Mean±S.D. One-way analysis of variance (ANOVA) and Tukey's post hoc test was used to find out the significant difference between the means in SPSS 23.0. Statistical significance was accepted at $P<0.05$.

**3 Results**

**3.1 Relative gut length and gastro somatic index**

The RGL and Ga.SI values of three different fish species are represented in Figures 1a and 1b, respectively. The RGL value was found to be significantly ($P<0.05$) higher in *T. fasciata* (4.20±0.45), and lowest in *M. tengara* (0.55±0.11) among the three species. The Ga.SI indicates the fullness of the stomach. The highest Ga.SI value was recorded in *T. fasciata* (5.61±1.22), whereas this value was 4.69±1.28 and 4.95±1.12 for *P. sophore* and *M. tengara*, respectively, which indicates good feeding intensity of the three species in the study.

**3.2 Gut content analysis**

The different food items observed in the gut of *T. fasciata, P. sophore,* and *M. tengara* fishes are shown in Table 1. All the observations on the three fishes revealed that food particles in the gut continuously lost their morphology and identity due to digestion as they passed down the digestive tract. In the posterior portion of the digestive tract, food was completely digested, and the undigested portion of the food started forming fecal matter. The gut content of *M. tengara* was observed to mainly consist of small crustaceans, rotifers, copepods, partially digested small invertebrates like head parts of shrimp, parts of insect larvae, appendages, wings,

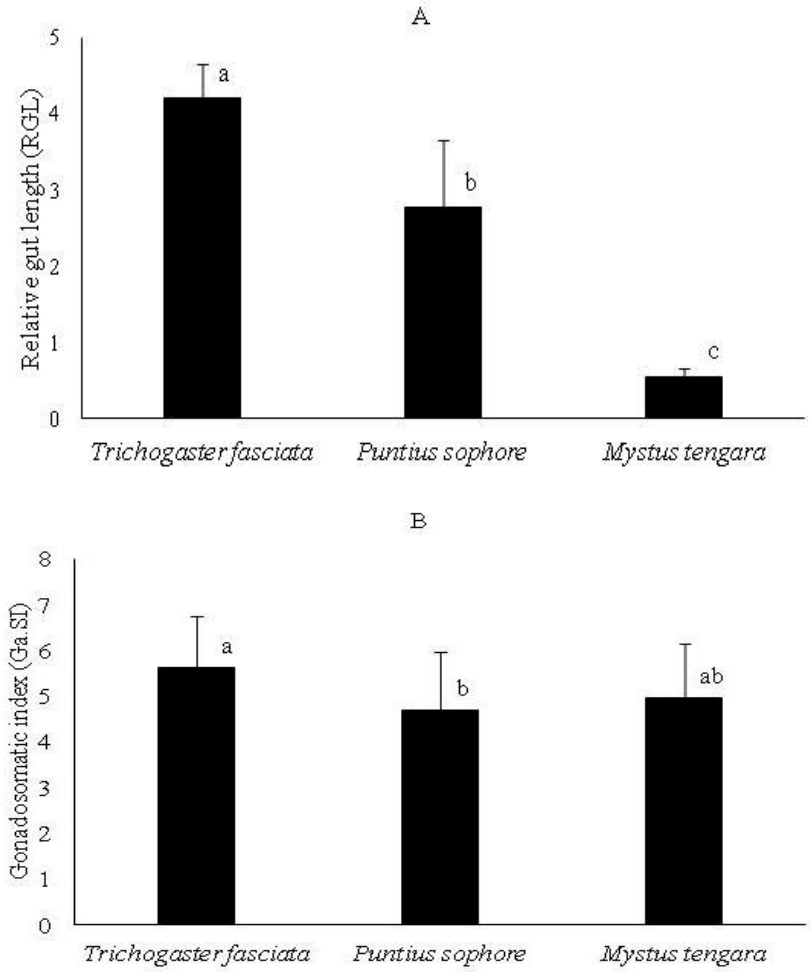



Figure 1 Relative gut length (RGL) and Gastro-somatic Index (Ga.SI) values of the three fish species studied. A: RGL. B: Ga.SI values. Values are represented as mean values ± SD (n=20). Means with different superscripts are significantly different ($P$<0.05).

Table 1 Composition of the gut content of the three fish species studied

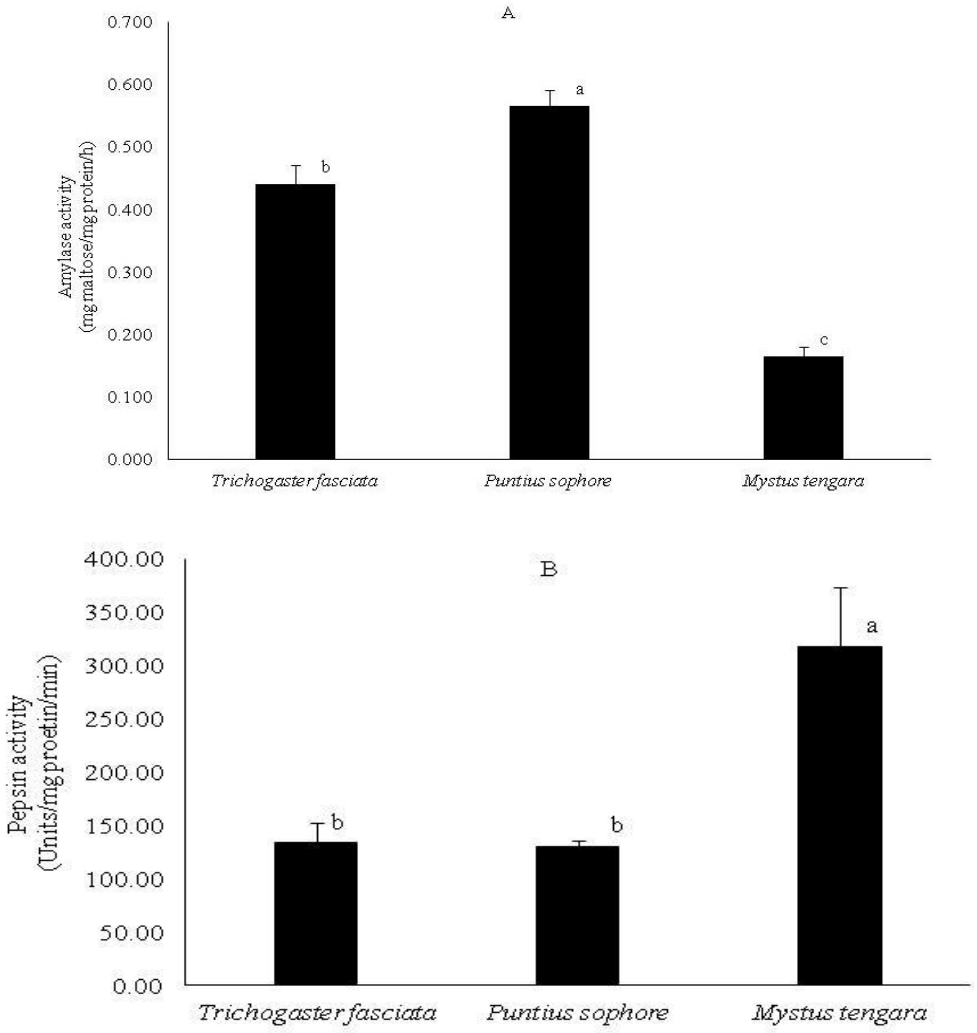
| Sl. No. | Feed Item | *T. fasciata* | *M. tengara* | *P. sophore* |
|---|---|---|---|---|
| 1. | Phytoplankton (*Oscillatoria spp., Spirulina spp., Rivularia spp., Achnanthes spp., Cymbella spp., Navicula spp., Tabellaria spp., Chlorella spp., Ulothrix spp., Oedogonium spp., Zygema spp.*) | + | - | + |
| 2. | Zooplankton (Crustaceans and their larval forms, *Daphnia spp.*, *Ceriodaphnia spp.*,) | + | + | + |
| | *Cyclops spp.*, | + | - | + |
| | Rotifers, *Brachionus spp.*, *Asplanchna spp*,) | + | - | + |
| 3. | Insects (Larvae, Pupa, Nymph, Adult, Exoskeleton, Appendages, etc.) | + | + | + |
| 4. | Macrophytes | - | - | - |
| 5. | Small fish and its parts | - | + | - |
| 6. | Unidentified material | + | + | + |
| 7. | Nematodes | - | - | + |

+ indicates presence and – indicates absence

and exoskeleton. No recognizable phytoplankton or plant-derived material was observed in its gut. The gut contents of *T. fasciata* consist of diatoms, phytoplankton, zooplankton, insect exoskeleton, and insect larvae, and therefore, the fish may be described as an omnivore in nature. Similar observations were also made in the gut content of *P. sophore* where phytoplanktons, zooplanktons, and small insect larvae were observed. Small nematodes were also observed in some of the specimens. The presence of unrecognizable organic and inorganic matter was detected in both the sample, however, it was more prominent in the case of *P. sophore*.

### 3.3 Digestive enzyme activities

Digestive enzymes showed species-specific variation in our study. The amylase activities in the three species were estimated, and the activity was significantly ($P$<0.05) higher in *P. sophore* (0.57±0.03 mg maltose/mg protein/h) among the three species (Figure 2a). *M. tengara* was found to have the lowest amylase activity (0.17±0.02 mg maltose/mg protein/h) among the three species studied, while *T. fasciata* recorded intermediate amylase activity between the other two species. The acid protease pepsin activity was found to be significantly ($P$<0.05) higher in *M. tengara* (317.45±55.95 Units/mg protein/min) compared to the other two species (Figure 2b). Pepsin activity was lower in *P. sophore* and *T. fasciata* compared to *M. tengara* but it did not differ significantly ($P$>0.05) between the two species. The highest total protease activity was observed in *M. tengara* (2.23±0.23 Units/mg protein/min) among three species. The activity in *P. sophore* and *T. fasciata* were 2.13±0.02 Units/mg protein/min and 1.23±0.08 Units/mg protein/min, respectively (Figure 2c). The activity of serine protease trypsin was significantly ($P$<0.05) higher in *P. sophore* (1.89±0.17 Units/mg protein/min) compared to the other species (Figure 2d). The lowest trypsin activity was recorded in *T. fasciata* (0.78±0.03 Units/mg protein/min) and the activity was intermediate in *M. tengara*.



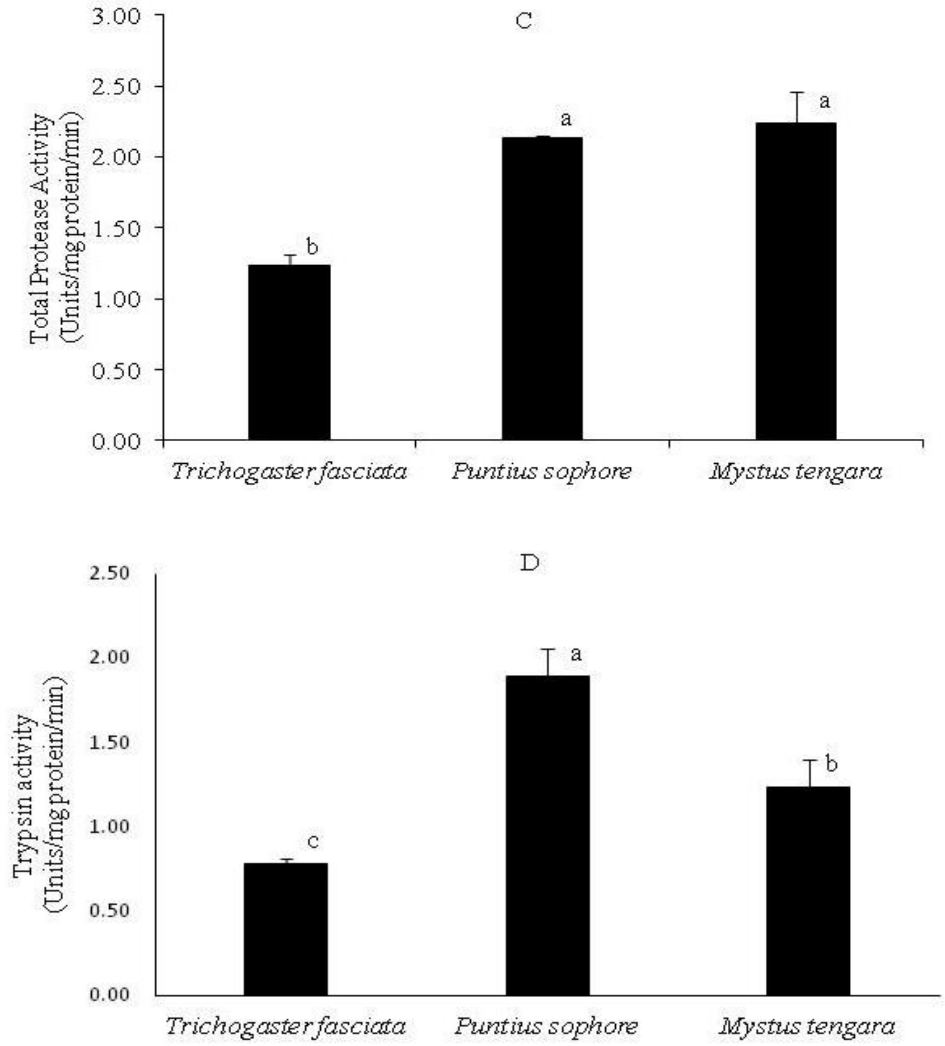



Figure 2 Digestive enzyme activity observed in the three fish species, *T. fasciata*, *P.sophore*, and *M. tengara*. A: Amylase activity. B: Pepsin activity. C: Total protease activity. D: Trypsin activity. Values are represented as mean±SD (n=10). Means with different lower case letters are significantly different ($P$<0.05).

## 4 Discussions

### 4.1 RGL and GaSI

Fish species have been classified as carnivores, herbivores, and omnivores by using the RGL as a main morphological variable while Ga.SI indicates feeding intensity (Manorama and Ramanujan 2017). Ga.SI values of all the three species in the present study indicate a good feeding intensity and these results were found to agree with earlier reports (Khongngain et al. 2017). Generally, when a fish species has the values of RGL less than 1, it is considered carnivorous in food habit, while 1-3 can be considered omnivore in nature. Herbivorous fishes having a plant or detritus-based diet are known to show RGL above 3 (Alam et al. 2019). In the present study, the higher RGL in *T. fasciata* and *P. sophore* reflects the presence of a relatively longer digestive tract compared to its body length. This allows the food to stay in the gut for a longer period, probably resulting in a more efficient mechanism for digestion and absorption, usually observed in animals with a diet rich in plant materials. The gut content analysis of the two species showed the presence of plant-derived materials in the present study. *M. tengara* with an RGL value of less than 1 indicates a highly carnivorous and predaceous feeding habit, which was also observed in its gut content analysis. Similar observations were made by Gupta (2004) where the RGL value was found to be 0.7, 3.7, and 4.7 for carnivorous, planktivorous, and herbivorous fishes, respectively, showing an increase with the increase in plant matter and a decrease with animal matter in the gut of the fish. RGL is closely related to the nature of food present in the fish gut (Khongngain et al. 2017). Plasticity of RGL under influence of diet

has been reported in some fish. RGL was low in younger *P. Ticto* and higher in adult individuals indicating their carni-omnivorous and herbi-omnivorous feeding nature, respectively (Koundal et al. 2012). Lanthaimeilu and Bhattacharjee (2018) also reported an increase in the RGL of *T. fasciata* with increasing size of the fish, indicating a shift in the feeding habit of the fish from carni-omnivore to herbi-omnivore.

**4.2 Gut content analysis**

The gut content analysis revealed the different feeding habits of the three species. The presence of exclusive animal-derived food and the absence of plant materials indicates the carnivorous and predatory nature of *M. tengara*. Similar results were reported by Gupta and Banerjee (2014), where zooplankton and rotifers were found as the most preferred food of *M. tengara* making it a carnivorous fish. In two related catfish species *M. seenghala* and *Wallago attu*, it was also observed that about 80-90% of animal food matter contributed to their gut content (Babare et al. 2013). However, a euryphagus omnivore feeding habit was also reported in *M. tengara* (Rao 2017) and a related species *M. gulio* (Begum et al. 2009; Sabbir et al. 2017) where the fish were found to feed on a wide range of food organisms.

The presence of a mixture of both plant-derived and animal-derived food materials in *T. fasciata* and *P. sophore* in the present study indicates their natural preference and also their plasticity in feeding habits. Such plasticity and flexibility in the diet preference may be an important reason for the wide distribution and success of these species. Similar plasticity in feeding habits was observed in an invasive mosquitofish (*Gambusia holbrooki*) from Italy and Spain (Pirroni et al. 2021). The omnivorous nature of *T. fasciata* and *P. sophore* has also been reported by previous researchers (Gupta 2004; Das and Kalita 2006; Khongngain et al. 2017). Our results of the gut content analysis of *P. sophore* agree with some earlier studies (Das et al. 2013; Risal et al. 2019) and with a related species *P. sarana* (Hossain et al. 2012). The results of the gut content analysis and that of RGL indicate that *T. fasciata* can be classified as an omnivore, while *M. tengara* is a carnivore. *P. sophore* with RGL less than 3 was found to have a highly omnivorous feeding habit. Studies on the gut content revealed a valuable information about the nature of food and feeding habits of the fish species, and also the type of food material available to the animals in the food chain. Further, present study revealed different feed compositions and feeding habits of the three species which may partially be due to differences in morphology (Velasco-Reyes et al. 2022), feed availability, and size differences of the studied species (Alam et al. 2020). Identifying the gut content is necessary to understand the availability of food in the natural environment of the fish, which may be useful in fisheries management (Al-Zibdah and Odat 2007). This may be useful in exploring the potential of the fish for culture and also for developing conservation strategies.

**4.3 Digestive enzyme activities**

Food and feeding habits along with the gut functional morphology influence the absence or presence of the digestive enzyme in fish. Our study indicates high amylase activity in *P. sophore* and this may be due to the significant contribution of phytoplankton and plant-based food component in its diet. Gioda et al. (2017) also reported higher amylase and maltase activities in the herbivorous species and intermediate activities in omnivorous species. A close relationship between herbivorous or omnivorous feeding habits and higher amylase activity was also reported by Hidalgo et al. (1999). In the present study, low amylase activity in *M. tengara* may be associated with its high carnivore diet based on animal-derived food. However, higher activities of carbohydrases, proteolytic, and lipases have been reported in detritivores species compared to the omnivorous and carnivorous fishes in some studies (López-Vásquez et al. 2009; Odedeyi and Fagbenro 2010).

Proteases are proteolytic enzymes that catalyze the breakdown of the larger protein molecules into smaller fragments and eventually to their component amino acid. Lower protease activities are generally reported in herbivorous and omnivorous fish species compared to carnivorous species (Chan et al. 2004; Chaudhuri et al. 2012). In the current study, high protease activity was observed in *M. tengara* compared to others. Similarly lower activity of amylase and higher alkaline protease activities in carnivorous fish than in the omnivorous and herbivorous fish species was reported by Champasri et al. (2021). High proteases and trypsin activities observed in *M. tengara* in the present study are in agreement with findings in other carnivorous fish species (Gioda et al. 2017; Weinrauch et al. 2019). Pepsin is an acid protease normally associated with the stomach region. The high pepsin activity observed in *M. tengara* indicates the differentiation of a stomach region and the presence of high animal protein in its diet. Trypsin is a serine protease active in alkaline conditions and its activity in omnivorous fish species are generally reported higher compared to the carnivores (López-Vásquez et al. 2009).

Results of the current study in the three SIFs suggest that the digestive enzyme activity in fish is influenced by the ingested diet or feeding habits which agrees with earlier reports (Chan et al. 2004; Langeland et al. 2013; Solovyev et al. 2014; Almeida et al. 2018). Digestive enzyme activities are thought to reflect the feeding habits of the fish and its diet preference (Langeland et al. 2013). Knowledge of the feeding biology of species is incomplete without knowing the relation between food habits and digestive enzyme activities (Almeida et al. 2018). Our study of the three species is significant in understanding the natural feeding pattern of the three species. Variations in the digestive enzyme activity are usually reported among different fish species and similar observations were also made in our study. The combined information on the feeding habits and the activities of the digestive

enzymes may be vital in formulating an appropriate diet for the successful culture of the three fish species.

**Conclusions**

In conclusion, the present study has established useful information on the activity of the digestive enzymes, and feeding habits of the three SIFs (*P. sophore, T. fasciata*, and *M. tengara*) in their natural habitat in lower Assam. Results of our study indicate that *P. sophore* and *T. fasciata* are omnivores, whereas *M. tengara* showed carnivore-type feeding habits. The digestive enzyme activities in the three species were found to be influenced by the food and feeding habit of each species. Diet-specific variations in the activities of amylase and proteases were observed in the study. High amylase and protease including trypsin activity was observed in *P. sophore* corresponding to the presence of both plants and animal food in its diet. Low amylase activity and high proteases activity in *M. tengara* corresponds to a highly carnivore-type feeding habitat and a minimal plant-based diet. Pepsin activity was seen to be highest in *M. tengara*, indicating the differentiation of a stomach. The results from this study may be useful in a better understanding of the feeding habit, and the digestive physiology of the three fish species in their natural habitat. This information may be useful in developing a suitable feed formulation required for the mass culture and production of the three species.

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**Conflict of interest**

All the authors declare no conflict of interest.

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