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# Digestive enzyme activities in four diverse small indigenous fish species from Sareswar beel of Kokrajhar, Assam in Northeast India

**Bichitra Narzary, Sanraja Muchahary, Bronson Kumar Khangembam***

*Department of Zoology, Bodoland University, Rangalikhata, Debargaon, Kokrajhar, Assam, India*

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**Key words:** Digestive enzymes, Small indigenous fish species, Relative gut length, Gastro-somatic index, Sareswar beel, Kokrajhar

**Abstract**

Small indigenous fish species (SIFs) are important source of protein and micronutrients for the local population and hence considered as potential for aquaculture expansion. *Pethia conchonius*, *Glossogobius giuris*, *Nandus nandus* and *Trichogaster fasciata* are such SIFs found in Sareswar beel of Kokrajhar, Assam, India. This is an important beel with potential for development of fisheries in the region. Information on the food habit and digestive physiology are important in understanding the nutritional biology of a fish species essential for designing appropriate diet and feeding strategy for its successful culture. The present investigation aims to study and compare the feeding habit and digestive enzyme profile of these species. Relative gut length data suggested that *P. conchonius* and *T. fasciata* were herbivores, while *N. nandus* and *G. giuris* were omnivore and carnivore, respectively. Ga.SI varied in all the species (2.65 ± 1.39 to 4.66 ± 2.14) and the result indicated good or high feeding intensity in all the species. The highest amylase and lipase activity was observed in *T. fasciata,* and significantly lower activity was recorded in *G. giuris* and *N. nandus,* respectively. Pepsin was found to be highest in *N. nandus* while total protease was greatest in *P. conchonius*. Plasticity in the food intake in the natural environment seems to influence the enzyme activities. The present study has established vital information on the digestive enzyme properties and feeding nature of the four SIFs which may be useful in the development of suitable feed for their mass production for their successful culture.

***Corresponding Author:** Bronson Kumar Khangembam ✉ kbronson173@gmail.com

## Introduction

Small indigenous freshwater fish species (SIFs) are an important source of protein and other essential nutrients like minerals for the local population, especially those rural populations living in and around natural freshwater resources such as the rivers, lakes, wetlands and beels. SIFs are those species which attain a size of about 25 cm in adult age in their life span/cycle (Felts *et al.,* 1996), and the maximum diversity of SIFs in India is found in the northeast region (Duarah and Das, 2019). SIFs are known to be relatively cheaper in comparison to larger food finfishes, possess rich content of calcium, proteins, and vitamins (Larsen *et al.,* 2000; Bhutia *et al.,* 2021) and occupy an important place in human civilization from ancient times. Traditionally, most of these SIFs are also consumed for their health benefits including disease healing properties by different tribes of the Northeast India. Some studies have also reported the application of various SIFs in the health care management (Duarah and Das, 2019).

The state of Assam in Northeast India is located between 21.57° N - 29.30° N latitude and 89.46° E - 97.30° E longitude covering an area of 78,438 km². The state is blessed with abundant water resources which include 5.49 lakh hectares of rivers like the mighty Brahmaputra, Barak etc., various wetlands (beels), ponds and low laying water bodies (Gogoi *et al.,* 2015). Sareswar beel in Kokrajhar District of lower Assam is a beel with immense potential for development of fisheries and aquaculture. These water resources are a rich source of many SIFs in the area. Majority of these indigenous fish species of Assam have high ornamental value and food value with high market price (Kalita and Deka, 2013; Baro *et al.,* 2014; Borah *et al.,* 2017). *Pethia conchonius* (Cyprinidae), *Glossogobius giuris* (Gobiidae), *Nandus nandus* (Nandidae) and *Trichogaster fasciata* (Osphronemidae) are such SIFs which have great potentialities in Assam (Baishya *et al.,* 2016; Bhuyan, 2016) as they possess good nutritive value and also in high demand. These are also preserved as fermented food known as *Shidol* or *Napham* in Assam by different ethnic communities (Kohinoor *et al.,* 2001; Kakati and Goswami, 2013; Mahanty *et al.,* 2014; Narzary *et al.,* 2016).

With the increasing pressure on higher production of fish for feeding the growing population, SIFs may be regarded as potential species for aquaculture expansion through sustained culture techniques as they are nutrient-rich and readily accepted by the people (Goswami, 2007; Roy *et al.,* 2022). Poly culture of these species can also enhance the income and livelihood of the fish farmers in the developing countries (Rajts *et al.,* 2022; Yengkokpam *et al.,* 2022). However, limited studies are available on the nutritional biology of these SIFs at present. Most of the studies available are related to the diversity and morphology of these species. For development of a successful culture technique, a better understanding of its nutritional physiology, feeding habit and digestive physiology is essential.

Proper understanding of the feeding habits helps in determining the nutritional requirement of the species (Emmanuel *et al.,* 2019) which is vital for its successful culture (Khan *et al.,* 2022), and also for the exploitation of the fish stocks (Meshram *et al.,* 2022). Studies on the digestive enzyme activities are important in comprehending the feeding biology of a fish (Almeida *et al.,* 2018) required for designing a proper feed and feeding strategy for its successful culture. Therefore, the aim of the present study is to evaluate the RGL, Ga.SI and digestive enzyme activities in the four important SIFs, *P. conchonius*, *G. giuris*, *N. nandus* and *T. fasciata* found in Sareswar Beel of Kokrajhar, Assam.

## Materials and methods

*Study site*

The study was conducted in a freshwater beel known as the Sareswar beel in Kokrajhar district of lower Assam ($26^0$8'31.3728" N, $89^0$55'11.208" E). Three different landing sites (LS1 - Lat 26° 8'14.12"N Long 89°55'45.89"E), (LS2 - Lat 26° 8'1.30"N Long 89°55'56.22"E) and (LS3 - Lat 26° 7'52.29"N Long 89°56'22.65"E) were chosen for sample collection (Fig. 1).

Covering an area of about 476 hectares, the beel is connected with Brahmaputra River *via* the Gadadhar River and is surrounded by dense Sal Forest and Rupshi Airport in the west, Sareswar village in the South, Bannyaguri village in the East, and Bashbari village in the North.

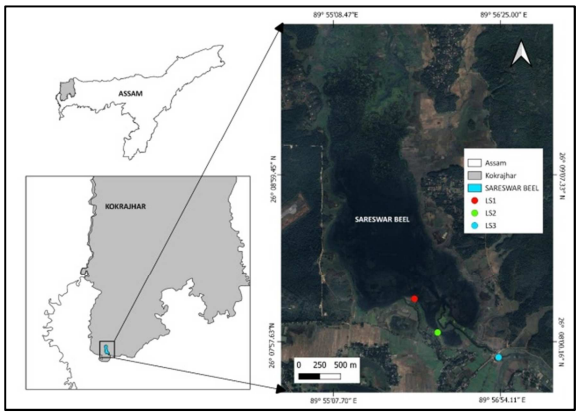



**Fig. 1.** Map of the study area showing Sareswar beel in Kokrajhar district of Assam, India.

*Sample collection*

Fifty adult individuals of each fish species (*P. conchonius*, *G. giuris*, *N. nandus* and *T. fasciata*) were collected during retreating monsoon season (September-November, 2022). Samples were collected using gill nets, scooping nets, trapping nets, etc. with the help local fishermen and brought to the laboratory at the Department of Zoology, Bodoland University, Kokrajhar, Assam. The fish sample were identified with the help of standard references (Talwar and Jhingran, 1991; Viswanath *et al.,* 2017; Froese and Pauly, 2021). The total weight and total length were also recorded (Asadi *et al.,* 2017). The representatives of each fish species were preserved in 10% formalin for future reference. For digestive enzyme study samples of the four species were collected live and transported to the Department of Zoology, Bodoland University for further study.

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

The freshly collected fish samples were washed and dissected on an ice-cold platform to collect the digestive tracts. The length and weight of dissected gastrointestinal tracts were measured to up to two decimal places. The relative gut length and the gastro-somatic index (Ga.SI) were calculated following Al-Hussaini (1949), and Bhatnagar and Karamchandani (1970), respectively using the following formulae:

RGL= Total length of gut / Total length of fish

Ga.SI = (Weight of gut × 100) / Weight of fish

*Digestive enzyme Activity*

*Preparation of crude enzyme extract*

Ten live individuals of each species *viz.*, *P. conchonius* (length: 8.23 ± 0.61cm, weight: 9.60 ± 2.02g), *G. giuris* (length: 11.64 ± 1.03cm, weight: 13.59 ± 4.51g), *N. nandus* (length: 11.53 ± 1.37cm, weight: 24.38 ± 10.06g) and *T. fasciata* (length: 8.80 ±1.09cm, weight: 12.12 ± 4.14g) were anesthetized with MS-222 (Tricaine methanesulfonate) and dissected on an ice-cold platform (0-4 °C). The digestive tracts of each sample were cleaned and weighed. Samples of the same species were pooled together, homogenized in cold distilled water (1:10 w/v, tissue: water) and centrifuged (Eppendorf 5425R, Germany) at 10,000 × g for 15 min at 4°C. The supernatants were collected and named as the crude enzyme and stored at -20°C for the further study.

*Amylase activity*

Amylase activity was measured following method of Bernfeld (1955), where 1% w/v starch solution was used as a substrate. The reaction mixture consisting of the crude enzyme extract, 1% starch in phosphate buffer (0.1 M, pH 7.0), and 1% NaCl was incubated for 1 hour at 37 °C. The reaction was stopped by using 3, 5- DNSA (3, 5-dinitro salicylic acid) and the changes in absorbance was recorded at 540nm (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.

*Lipase activity*

The lipase activity was recorded by colorimetric evaluation of *p*-nitrophenol released because of enzymatic hydrolysis of *p*-nitrophenyl palmitate (Sigma, St Louis, USA) at 410nm by the crude enzyme extract (Winkler and Stuckman, 1979).

Crude extract was incubated with the substrate solution for 15 mins at 37 °C and the change in absorbance was measured at 410nm. One enzyme unit was defined as 1µmol of *p*-nitrophenol (PNP) enzymatically released from the substrate $ml^{-1}$ $min^{-1}$.

*Pepsin activity*

Pepsin activity was measured by using haemoglobin as the substrate (Anson, 1938). Briefly, to 375 µl of 2% hemoglobin, 75 µl of crude extract was added and incubated at 37 °C for 10 minutes. The reaction was stopped by adding 500 µl Tricholoacetic acid (5% w/v), centrifuged (12,000 rpm, 5 mins at room temperature) and its absorbance was measured at 280 nm. Specific pepsin activity was measured using the formula:

Activity = Abs (test-blank) × 1000/mg protein/min.

*Total protease activity*

Total protease activity was measured following the method described by Garcia-Carreno (1992) by using azocaesin (in 50 mM Tris-HCl, pH 7.5, SRL, India) as a substrate. The reaction mixture consisting of the crude extract, substrate (azocaesin) and buffer was incubated for 10 mins at 25 °C and the reaction was terminated by adding 20% TCA. The samples were centrifuged (10,000rpm, 5 mins, 20 °C) and the absorbance was recorded at 366 nm using a spectrophotometer (UV-Visible Spectrophotometer, Shimadzu 1900i, Japan). Specific total protease activity was expressed in Units mg $protein^{-1}$ $min^{-1}$.

*Protein estimation*

Total soluble protein was measured using the method of Bradford (1976). BSA (Bovine Serum Albumin, 1 mg $ml^{-1}$) was used as the standard for measuring protein.

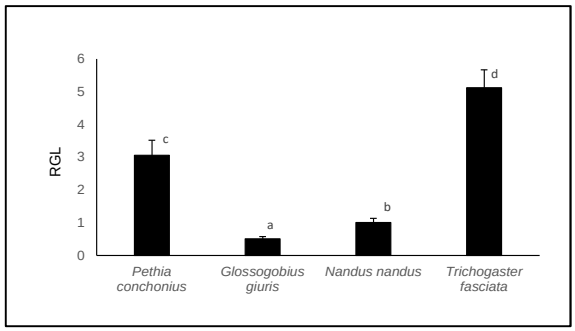
*Statistical Analysis*

All data of length, weight, RGL, Ga.SI and digestive enzyme activities were represented as mean ± SD. One-way analysis of variance (ANOVA) and Tukey's post hoc test was used to test the significance difference between the means using SPSS 23.0. The level of statistical significance was accepted at $P < 0.05$.

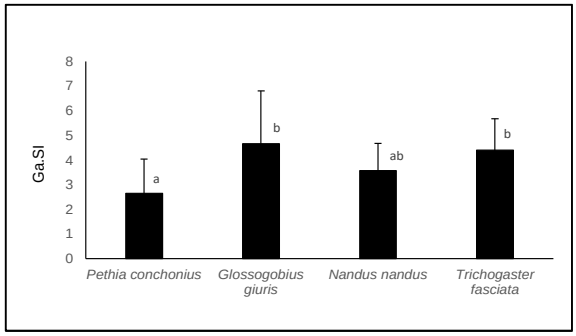
**Results and discussion**

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

The RGL and Ga.SI values of four different indigenous fish species are shown in figures (Fig. 2a, b). The highest RGL value was found highest in *T. fasciata* (5.12 ± 0.55) followed by *P. conchonius* (3.06 ± 0.45) and *N. nandus* (1.01 ± 0.12), while *G. giuris* (0.51 ± 0.07) showed the lowest RGL value. The Ga.SI value indicates the feeding intensity of the species and the highest feeding intensity was recorded in *G. giuris* (4.66 ± 2.14) and *T. fasciata* (4.40 ± 1.27). The Ga.SI of *N. nandus* (3.57 ± 1.10) and *P. conchonius* (2.65 ± 1.39) also indicated a high feeding intensity in the habitat during the study period.




**(a)**




**(b)**

**Fig. 2.** (a) The relative gut length (RGL) and (b) Gonadosomatic Index (Ga.SI) of the four experimental fish species *viz. Pethia conchonius, Glossobius giuris, Nandus nandus* and *Trichogaster fasciata*. Values are represented as mean values ± SD. Bars with different superscript are significantly different ($P < 0.05$).

The RGL is an important indicator of the feed and feeding habit of a fish species. A species can be considered as herbivores, omnivores and carnivores

by using the RGL and analysing the gut contents. The present study showed that the four different species under investigation indicated different feeding habit based on the data from RGL. The RGL values are generally low in carnivores, high in omnivorous and highest among the three in herbivorous (Das and Moitra, 1963; Manorama and Ramanujam, 2017). RGL in carnivorous fishes are usually reported to be less than 1, while its value of 1-3 and > 3 can be considered as omnivore and herbivore (plants or detritus food habits), respectively (Singh *et al.,* 2018; Alam *et al.,* 2019). In the present study, *G. giuris* with an RGL of 0.51 ± 0.07 indicated a highly carnivorous feeding habit, while *N. nandus* may be considered having an omnivorous feeding habit as its RGL was found to be 1.01 ± 0.12. In a similar result, *G. giuris* was reported as carnivorous and food items of digestive gut consist of insects, fish, crustaceans and zooplanktons (Hossain *et al.,* 2016).

Our result also agrees with Goswami (2007) in which the presence of organic food like prawns, insects, macrophytes, zooplankton, etc., in the digestive gut of *N. nandus* was reported indicating the omnivorous nature of the species. The RGL in *N. nandus* (1.01 ± 0.12) observed in the present study indicated an omnivorous feeding habit. Similar range of RGL (1.28 ± 0.10 to 1.58 ± 0.04) was also observed by Manorama and Ramanujam (2017) in an omnivorous fish species, *P. shalynius*. The RGL value of *P. conchonius* and *T. fasciata* were found to be 3.06 ± 0.45 and 5.12 ± 0.55 in our study, indicating herbivorous feeding habit depending mostly on plant derived food materials. Similar high RGL were also reported by Roy *et al.* (2022) for *T. fasciata* from Assam. However, some studies also suggested omnivorous feeding habit of *T. fasciata* (Das and Moitra, 1963; Das and Kalita, 2006). This indicated flexibility in the feeding habit of the species in different feeding environment. Lanthaimeilu and Bhattacharjee (2018) also, reported that *P. conchonius* and *T. fasciata* changes their feeding habits from Carni-omnivorous to Herbi-omnivorous with increase in its size.

The Ga.SI generally shows the feeding intensity of the fish species in their life cycle within their habitat (Sangma *et al.,* 2019; Tran *et al.,* 2021). Hence, this index can be an indirect indicator of the food availability of a particular species in the area. All the four species in this study showed high feeding intensity as indicated by their high values of Ga.SI, which also shows the abundance of their natural feed in the beel during the study period. The Ga.SI of *T. fasciata* (4.40 ± 1.27) in the present study was found to be in the range (0.104 to 5.655) described by Khongngain *et al.* (2017) for the same species. The Ga.SI of *P. conchonius* reported in this study strongly agrees with the range of 1.08-3.03 observed by Lanthaimeilu and Bhattacharjee (2018) in the same species. However, the Ga.SI has been reported to show variation for the same species in different seasons reflecting the feeding intensity. For instance, Singh *et al.* (2018) reported that the Ga.SI of the Giant river-catfish *A. seenghala* showed variation in different month which may be due to entry of different phases of life.

A distinct reduction of Ga.SI from the month of July to March (6.28-3.42) was reported by Naik *et al.* (2015) in *C. carpio* where Ga.SI value was found high during warmer months and gradual decline occurs in approach to winter. Dinh *et al.* (2018) also described the seasonal variation of feeding intensity in *P. septemradiatus* (mudskipper). These changes may be helpful in understanding the fish's adaptation in a changing environment. Similar report of variation in Ga.SI value (2.13-3.14) was described by Kurbah and Bhuyan (2018) in *M. chucia* in different season of their life. *C. nama* and *T. lalius* also adapted different feeding intensity in different months of a year in their habitat (Sangma *et al.,* 2019). Hence, a comparative study in the Ga.SI and RGL for different seasons may be useful for understanding the dynamics of the feeding habit of the species under study.

*Digestive enzyme activities*

Digestive enzymes study indicated a species-specific variation in our investigation (Table 1). The amylase activity in four different species were 2.67 ± 0.07,

1.38 ± 0.05, 1.36 ± 0.01 and 1.01 ± 0.05 mg maltose mg protein$^{-1}$ h$^{-1}$ in *T. fasciata*, *P. conchonius*, *N. nandus* and *G. giuris,* respectively. Significantly ($P$ < 0.05) lowest amylase activity was observed in in *G. giuris* out of the four species studied while the highest was observed in *T. fasciata*. However, *P. conchonius* and *N. nandus* showed no significant difference ($P$ > 0.05) in their amylase activity. Highest lipase activity was found in *T. fasciata* (0.26 ± 0.00 µM PNP ml$^{-1}$ min$^{-1}$, $P$ < 0.05) and the lowest was recorded in *N. nandus* (0.11 ± 0.01 µM PNP ml$^{-1}$ min$^{-1}$). The lipase activities were 0.21 ± 0.00 and 0.15 ± 0.00µM PNP ml$^{-1}$ min$^{-1}$ in *P. conchonius* and *G. giuris,* respectively.

The acid protease pepsin activity ranged from 349.60 ± 6.79 to 926.80 ± 89.78 units mg protein$^{-1}$ min$^{-1}$ in the four species. Significantly higher ($P$ < 0.05) pepsin activity was recorded in *N. nandus* (926.80 ± 89.78 units mg protein$^{-1}$ min$^{-1}$) in comparison to other three species. The total protease activity was found significantly ($P$ < 0.05) highest in *P. conchonius* (13.67 ± 1.05 units$^{-1}$ mg protein$^{-1}$ min) among the four species in the present study. *N. nandus* recorded the lowest total protease activity (0.71 ± 0.12 units mg protein$^{-1}$ min$^{-1}$), while the activities were 3.93 ± 0.27 and 1.23 ± 0.12 units mg protein$^{-1}$ min$^{-1}$ in *T. fasciata* and *G. giuris*, respectively.

**Table 1.** Digestive enzyme activity of different species

| Species | Digestive enzyme activity | | | |
|---|---|---|---|---|
| | Amylase (mg maltose mg protein$^{-1}$ h$^{-1}$ | Lipase (µM PNP ml$^{-1}$ min$^{-1}$) | Pepsin (units mg protein$^{-1}$ min$^{-1}$) | Total Protease (units mg protein$^{-1}$ min$^{-1}$) |
| *Pethia conchonius* | 1.38 ± 0.05$^{b}$ | 0.21 ± 0.00$^{c}$ | 416.67 ± 49.42$^{a}$ | 13.67 ± 1.05$^{c}$ |
| *Glossogobius giuris* | 1.01 ± 0.05$^{a}$ | 0.15 ± 0.00$^{b}$ | 349.60 ± 6.79$^{a}$ | 1.23 ± 0.19$^{a}$ |
| *Nandus nandus* | 1.36 ± 0.01$^{b}$ | 0.11 ± 0.01$^{a}$ | 926.80 ± 89.78$^{b}$ | 0.71 ± 0.12$^{a}$ |
| *Trichogaster fasciata* | 2.67 ± 0.07$^{c}$ | 0.26 ± 0.00$^{d}$ | 398.53 ± 29.61$^{a}$ | 3.93 ± 0.27$^{b}$ |

Values are represented as mean values ± SD. Means within the same column having different superscripts are significantly different ($P$ < 0.05).

The digestive enzyme activities are influenced by the feeding behaviour, and are often considered a health index for a species. Four important digestive enzyme activities were evaluated in the selected species. Our results showed the highest amylase activity in *T. fasciata* and intermediate activities in *P. conchonius* and *N. nandus*, which may be attributed to their herbivorous or omnivorous feeding habit comprising significant amount of phytoplankton and other plant-derived food components in their diet. Similar results were reported by Gioda *et al.* (2017) and Roy *et al.* (2022) where higher amylase activities were observed in hervivorous fishes compared to carnivores.

*G. giuris* showed significantly ($P$ < 0.05) lowest amylase activity among the four species which may attributed to its carnivorous feeding habit and a lower secretion of the enzyme required for hydrolysis of plant derived complex carbohydrates. Pepsin is an acid protease associated with the stomach and its helps in hydrolysis of proteins at acidic pH. *N. nandus* and *P. conchonius* showed the high pepsin activity among the four species which indicates the presence of active gastric cells secreting the enzyme. High pepsin activity may also be correlated with a diet rich in protein.

Protease enzyme catalyses the breakdown of complex protein molecules into simpler units. In the present study, highest total protease activity was observed in the herbivorous species *P. conchonius* and *T. fasciata* than omnivorous and carnivorous species. Similar results were reported by Hlophe *et al.* (2014), where the highest protease activity was observed in tilapias (*T. rendalli* and *O. mossambicus*) eating plant derived food than an omnivore fish. The prominent protease activity may be an adaptive advancement to efficiently employ the low protein content diet in their environment. The higher protease activity in fishes which mainly preferred diet of plant origin than in those depending on animal diets were also reported by Chaudhuri *et al.* (2012).

Enhanced protease activity in herbivorous fish may also be due to increased rate of food consumption to make up for the plant based lower protein diets (Hofer, 1982).

Lipase activity was detected in all the four species which indicated the ability of the four species to hydrolyse complex lipids in their diet. Results of our study showed high lipase activity in *P. conchonius* and *T. fasciata* than omnivore (*N. nandus*) and carnivore species (*G. giuris*). Reports on the activity of lipase are diverse and often inconsistent in various studies. Carnivorous fishes are usually reported with high lipase activity than carnivorous (Langeland *et al.,* 2013). However, in another study, intense lipase activity was reported in the herbivore fish Nile tilapia (*O. niloticus*) by Tengjaroenku *et al.* (2000). Again, a stomach less insectivorous fish *Z. buffonis* was reported to have increased lipase activity during digestion (Abidin *et al.,* 2016). These variations in many enzyme activities in fish may be due to the changes in feeding habit with age, gradually shifting from carnivorous to herbivorous feeding nature (Pujante *et al.,* 2017).

Various studies show that the activities of digestive enzymes in fish are influenced by the food and feeding habit in nature (Hidalgo, 1999; Tengjaroenkul, 2000; Lundstedt *et al.,* 2003). Those species, which are known for wide spectrum of dietary preferences in their environment, may also increase the variety of enzyme function. Hence, the variation of enzyme activities in the present study may be due to the difference in species, diet and local adaptation both spatial and temporal. Presence of important enzymes hydrolysing carbohydrates, proteins and lipids, and their degree of variations were observed in this study for the four SIFs. These data may be useful for designing appropriate feed and feeding strategies for the species.

## Conclusions

The present study has established important characteristics of the digestive enzymes and feeding habits of the four SIFs, *P. conchonius*, G. giuris, *N. nandus* and *T. fasciata* found in Sareswar beel of Kokrajhar, Assam for the first time. Our study on the RGL index showed that *P. conchonius* and *T. fasciata* are herbivorous, whereas *N. nandus* and *G. giuris* exhibited omnivorous and carnivorous feeding habit, respectively. Food and feeding nature of the four different species seems to influence the digestive enzyme activity in the four species. Enzymes hydrolysing carbohydrates, proteins and lipids were detected in all the species but with variations probably complementing their different feeding habits. This study may be useful in understanding the digestive physiology of the four species in the Sareswar beel of Assam. This information may be beneficial for the development of their feed and feeding strategy based on the digestive physiology of the four species for their mass production.

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## Conflict of interest

The authors declare no conflict of interest

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