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Analysis of Digestive Enzymes Concentration in Gut of Juvenile and Adult Group of *Notopterus notopterus* (Notopteridae; Osteoglossiformes)

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Analyzing digestive enzyme concentration in the gut is important to know about the digestive physiology of fish. Therefore, we determined concentrations of protease, lipase, and amylase enzyme in gut extracts of juvenile and adult groups of *Notopterus notopterus* relative to total body length (TBL), body weight (W), gut length (GL), relative gut length (RGL), Zihler's index (ZI) and condition factor (K). A total of 60 *N. notopterus* specimens (30 juvenile and 30 adult) were collected, the gut of each removed, homogenized, and evaluated for the concentrations of protease, lipase, and amylase. Protease and lipase concentration were observed significantly ($P < 0.01$) correlated with increased TBL, W, and GL in both juvenile and adult groups of *N. notopterus*. Amylase concentration significantly ($P < 0.01$) increases with an increase in TBL, weight, and GL in the juvenile group, whereas amylase concentration significantly ($P < 0.01$) decreases with an increase in TBL, W, and GL in the adult group. K had a significant ($P < 0.01$) positive correlation with protease and lipase concentrations in both juvenile and adult groups, whereas K showed a significant ($P < 0.01$) positive correlation with amylase concentrations in juvenile and significant ($P < 0.01$) negative correlation in the adult group. Protease and lipase concentration were observed significantly ($P < 0.01$) correlated with an increase in ZI in the juvenile and adult groups. Amylase concentration significantly ($P < 0.01$) increases with an increase in ZI in the juvenile group, whereas amylase concentration significantly ($P < 0.01$) decreases with an increase in ZI in the adult group. The concentrations of protease (344.12 U/mg) and lipase (172.49 U/mg) were observed high in juvenile and adult groups, whereas amylase concentration (3.80 U/mg) was found low in the juvenile and adult *N. notopterus* groups. Present study results revealed the digestive physiology and carnivorous behavior of *N. notopterus*. Moreover, information from the present study about digestive enzyme concentration in juvenile and adult species of *N. notopterus* provides valuable information to understand the digestive physiology of carnivorous fishes.

Keywords: amylase, carnivorous fish, lipase, *Notopterus notopterus*, protease

INTRODUCTION

Animal growth not only depends on its feed and nutritive profile but also on its ability to ingest, digest, absorb, and metabolize the nutrients of feed. In aquatic animals, nutrient utilization depends on the concentration of digestive enzymes (Bhuyan *et al*. 2022; Nazir *et al*. 2023). The developmental stage and age influence the activity of digestive enzymes in different fishes (Kuz'mina 1996). Evaluation of digestive enzyme concentration provides the basis of feed formulation in terms of nutritive values and cost-effectiveness for different sizes of fish (Pujante *et al*. 2017). Nutrient digestion depends on the concentration of secreted enzymes from the digestive tract (Bhuyan *et al*. 2022). Generally, digestive enzyme concentration in

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the gut varies with feeding habits and gut morphology (Solovyev and Gisbert 2016).

Notopterus notopterus (Pallas, 1769) is a carnivorous fish belonging to the Notopteridae family. *N. notopterus* feeds on aquatic weeds, insects, and small fishes (Kiran and Waghray 1998; Shillewar and Nanware 2009). *N. notopterus* body length up to 27.5 cm is considered juvenile, whereas a body length of 28 cm and above is considered adult species (Yanwirsal *et al*. 2017). Though the concentration of digestive enzymes has been assessed in the gut of different carnivorous fish, to the best of our knowledge, there is no data on digestive enzyme concentration in different size groups of *N. notopterus* available until now. Therefore, in the present study, we determined the concentrations of digestive enzymes (amylase, lipase and proteases) in the gut of juvenile (size range 20.10–25.30 cm) and adult (size range 39.1–48.7 cm) groups of *N. notopterus* relative to total body length (TBL), body weight (W), gut length (GL), relative gut length (RGL), Zihler's index (ZI) and condition factor (K).

MATERIALS AND METHODS

A total of 60 specimens (30 juvenile and 30 adult) of *Notopterus notopterus* were collected from Balloki Headworks (71°51'42.7E, 31°13'12.5"N), Ravi River, Pakistan (Figure 1) during the year 2020.

The collected specimens were divided into juvenile and adult groups on the basis of their total body length (TBL). Each group contains 30 specimens of *N. notopterus* species. A mean body length of 22.63 ± 1.78 cm and a BW of 103.87 ± 31.45 g were recorded for the juvenile group, whereas a mean body length of 44.23 ± 3.38 cm and a BW of 394.96 ± 102.28 g were recorded for adult group of *N. notopterus*.

All experiments were performed according to the guidelines of the Advance Study and Research Board of Bahauddin Zakariya University, Multan, Pakistan. Before dissection, collected fish specimens were euthanized, following the method of Stoskopf (2015). The wild freshwater fish species euthanasia procedure handling was approved by the institutional animal care board. The dissection was carried out with sterile instruments (washed with 70% ethanol). Then, the complete gut was removed, its total length measured, and put into the sterile tube.

Enzyme Extraction and Concentration Analysis

The complete gut of each specimen was homogenized separately with chilled Tris. HCl 50 mM. Then homogenate was centrifuged at 6000 rpm × g at 4 °C for 15 min. After centrifugation, the supernatant was collected and put at –4 °C for further analysis (Ismat *et al*. 2013). Given formulas were used for the calculation of various indices:

Relative gut length (RGL) = Length of the intestine (cm)/ Total length of fish (cm) (Al-Hussaini 1949; Riaz and Naeem 2020; Ahmed *et al*. 2022)

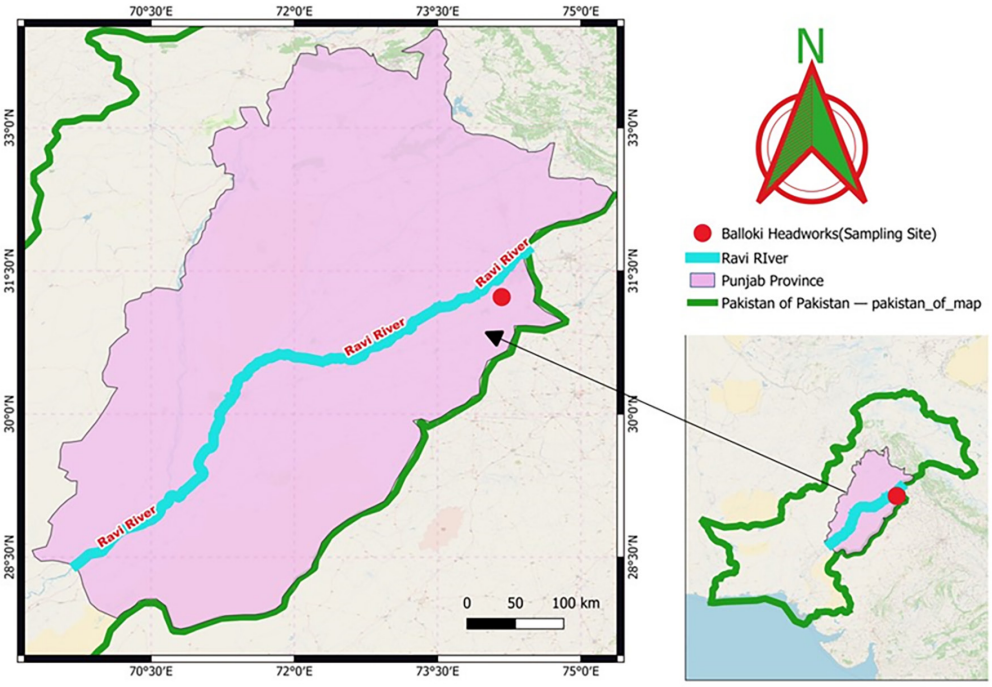

Figure 1. Pakistan map showing the study area and sample collection site of *Notopterus notopterus*. Balloki Headworks, Ravi River located at 71°51'42.7E, 31°13'12.5"N.

Zihler's index (ZI) = Total gut length (cm) x [Fish body mass (g) $1/3]^{-1}$ (Zihler 1982; Riaz and Naeem 2020; Ahmed *et al*. 2022)

Condition factor (K) = Fish body mass (g) x [Total length $(cm)^3]^{-1}$ x 100 (Htun-Han 1978, Datta *et al*. 2013; Riaz and Naeem 2020)

Amylase

Amylase concentration was evaluated by using starch as a substrate (Bernfeld 1955). One (1) mL gut homogenate and 1 mL (1%) starch substrate (soluble starch 1 g, NaCl in 100 mL, NaH_2PO_4 0.02 M, pH 6.9) were incubated at 37 °C for 3 min. Then, 2 mL of 3.5-dinitrosalicylic acid reagent was added, and the reaction was stopped. Then, the solution was heated for 5 min in a boiling water bath. Then, it was cooled and to which 20 mL of distilled water was added. Absorbance was checked at 540-nm wavelength using a spectrophotometer (Model Hitachi-U–2900) against a blank (Deguara *et al*. 2003; Ismat *et al*. 2013; Iqbal *et al*. 2016; Murtaza *et al*. 2016; Riaz and Naeem 2020).

Absorption of standard = A nm; Absorption of sample = Y nm

"A" absorption is due to = 1 mL (0.01 mg of standard glucose in 1-mL (glucose 10%) (1-mg glucose/ 10 mL) "1" is = 0.01/A

"Y" absorption is due to = (0.01/A) × Y = "F" mg of amylase in sample

Activity of amylase (F × 1000/ 180 (MW of glucose) ×15 = "Z" IU/mL (Ismat *et al*. 2013).

Lipases

One (1) mL of the gut homogenate sample and 3.5 mL of phosphate buffer (0.2 M, pH 6.9) were added into the flask. Then, 0.5 mL of olive oil was added into the phosphate buffer, and the flask was placed in a boiling water bath at 37 °C for 30 min and stirred. After that, 1 mL of acetic acid was added to the mixture, following which 3–4 drops of the phenolphthalein indicator were added to the enzyme mixture. Ten (10) mM NaOH was taken in the burette for titration. Then, the enzyme mixture was titrated using 10 mM NaOH until the pink color appeared (Worthington 1991; Deguara *et al*. 2003; Zamani *et al*. 2009; Ismat *et al*. 2013; Iqbal *et al*. 2016; Murtaza *et al*. 2016; Riaz and Naeem 2020).

Units/mL of enzyme = Volume of NaOH × Normality of NaOH × 40/ Volume of enzyme sample used = "Y" µM of oleic acid released min^{-1}

Now enzyme activity = Y × 1000/ 254 (MW oleic acid) × 30 min (Ismat *et al*. 2013)

Proteases

The concentration of protease was determined using 1% azocasein in 50 mmol tris HCl (pH 7.5) (Garcia-Carreno 1992). 10 µl enzyme extract was mixed in 0.5-mL buffer (Tris-HCl 50 mmol, pH 7.5). Then, a 0.5-mL substrate solution was added and incubated at 25 °C temperature for 10 min. Furthermore, the reaction was stopped by adding 0.5 mL of trichloro-acetic acid (20%). After that, it was centrifuged at 14000×g for 5 min. Then, the absorbance of the supernatant was recorded at 366 nm. A standard curve was prepared by using azocasein (Garcia-Carreno 1992; Ismat *et al*. 2013; Iqbal *et al*. 2016; Riaz and Naeem 2020; Lal *et al*. 2022).

Statistics Analysis

Statistical analysis was applied to the data by calculating the minimum, maximum, mean, and standard deviation values. Data on TBL, W, GL, RGL, ZI, and K were analyzed with appropriate regression model analysis and Pearson's correlation using Microsoft Excel 2016.

RESULTS

Analysis of Digestive Enzyme Concentration of Juvenile Group of *Notopterus notopterus*

Analysis of TBL, W, GL, RGL, ZI, K, and protease, lipase and amylase concentrations are provided in Table 1.

TBL showed significant ($P < 0.01$) positive correlation with protease, lipase, and amylase concentrations in the juvenile group (Table *2)*. The concentrations of protease, lipase, and amylase increased with increase in TBL in juvenile group of *N. notopterus* (Figure 2a).

The W exhibited significant ($P < 0.01$) positive correlation with protease, lipase, and amylase concentrations in the juvenile group (Table 3). The concentrations of protease, lipase, and amylase showed an increasing trend with increase in the total W in the juvenile group (Figure 3a).

RGL and GL showed significant ($P < 0.01$) positive correlation with protease, lipase, and amylase concentrations in the juvenile group (Table 4). The concentrations of protease, lipase, and amylase increased with increase RGL and GL in the juvenile group (Figure 4a).

K revealed a significant ($P < 0.01$) positive relation with the increasing pattern of protease, lipase, and amylase concentrations in the juvenile group (Table 5).

The protease, lipase, and amylase concentrations exhibited a significant ($P < 0.01$) positive correlation with an increase in ZI in the juvenile group (Table 6). Figure 5a indicated that protease, lipase, and amylase concentrations increased with an increase of ZI in the juvenile group.

Table 1. Analysis of gut morphometric parameters, digestive enzyme concentration, condition factor (K), and Zihler's index (ZI) in juvenile and adult group of *Notopterus notopterus*.

Body constituents	**Group 1: juvenile**		**Group 2: adult**	
	Range	Mean ± SD	Range	Mean ± SD
Total length (TL) cm	20.10–25.30 cm	22.63 ± 1.78	39.1–48.7 cm	44.23 ± 3.38
Log total length (TL)	1.30–1.40	1.35 ± 0.03	1.59–1.69	1.64 ± 0.03
Body weight (W) g	63.10–155.20 g	103.87 ± 31.45	251.1–547.3 g	394.96 ± 102.28
Log body weight (W)	1.80–2.19	2.00 ± 0.13	2.40–2.74	2.58 ± 0.12
Gut length (GL) cm	11.1–16.50 cm	13.78 ± 1.82	30.9–42.9 cm	37.08 ± 4.3
Log gut length	1.05–1.22	1.14 ± 0.06	1.49–1.63	1.57 ± 0.05
Relative gut length	0.55–0.65	0.61 ± 0.03	0.79–0.88	0.84 ± 0.03
Condition factor (K)	0.78–0.96	0.87 ± 0.06	0.42–0.47	0.45 ± 0.02
Zihler's index (ZI)	23.35–85.36	49.42 ± 21.16	258.63–782.64	501.26 ± 183.54
Protease concentration	78.41–178.12 U/mg	124.71 ± 33.57	150.41–344.12 U/mg	241.51 ± 64.58
Log protease concentration	1.89–2.25	2.08 ± 0.12	2.18–2.54	2.37 ± 0.12
Lipase concentration	85.04–94.49 U/mg	89.61 ± 3.23	160.04–172.49 U/mg	166.71 ± 4.01
Log lipase concentration	1.93–1.98	1.95 ± 0.02	2.20–2.24	2.22 ± 0.01
Amylase concentration	1.17–1.95 U/mg	1.56 ± 0.25	1.99–3.80 U/mg	2.83 ± 0.60
Log amylase concentration	0.07–0.29	0.19 ± 0.07	0.30–0.58	0.44 ± 0.09

[SD] standard deviation

Table 2. Regression analysis of log total body length correlation with digestive enzyme (protease, lipase, amylase) concentration in juvenile and adult group of *Notopterus notopterus*.

Relations	**Group 1: juvenile**						**Group 2: adult**					
	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t value, when b = 3	*P*-value	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error	t-value, when b = 3	*P*-value
Log TL (x) Log protease (y)	**0.991*****	0.762	0.283	0.004	57.819	**0.000001**	**0.989*****	0.985	0.278	0.005	50.255	**0.000005**
Log TL (x) Log lipase (y)	**0.989*****	–2.886	2.171	0.0420	50.742	**0.000004**	**0.982*****	–5.368	3.156	0.080	39.420	**0.000004**
Log TL (x) Log Amylase (y)	**0.854*****	1.273	0.427	0.033	12.815	**0.000003**	**0.976*****	1.802	–0.355	0.010	–34.214	**0.000002**

****P* < 0.01; [TL] total length

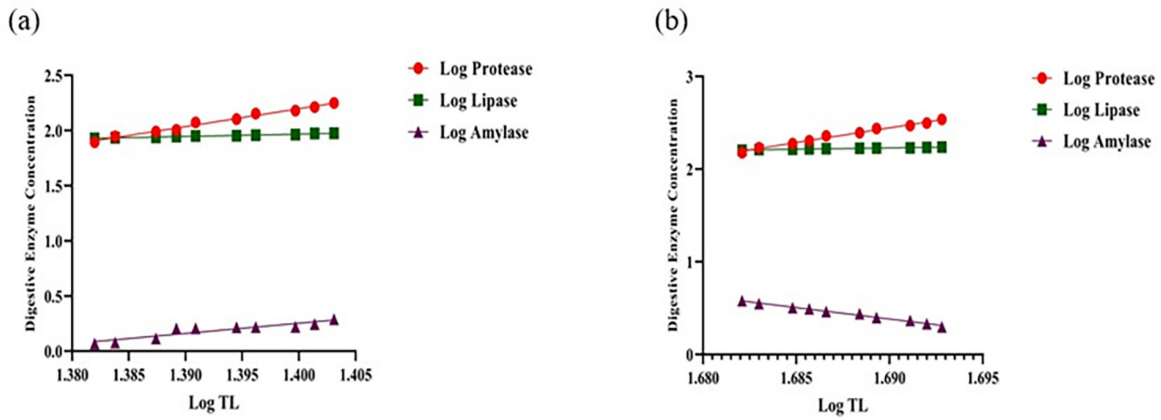

Figure 2. Correlation of log total body length with digestive enzyme (protease, lipase, amylase) concentration (mean) in [A] juvenile and [b] adult group of *Notopterus notopterus*.

Table 3. Regression analysis of log body weight correlation with digestive enzyme (protease, lipase, amylase) concentration in juvenile and adult group of *Notopterus notopterus*.

Relations	Group 1: juvenile						Group 2: adult					
	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error	t-value, when b = 1	*P*-value	R (correlation coefficient)	A (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 1	*P*-value
Log W (x) Log protease (y)	**0.991*****	–0.301	1.105	0.019	57.335	**0.000001**	**0.993*****	0.306	0.961	0.014	64.280	**0.000005**
Log W (x) Log lipase (y)	**0.990*****	–14.509	8.456	0.158	53.363	**0.000001**	**0.988*****	–21.675	10.918	0.226	48.277	**0.000001**
Log W (x) Log amylase (y)	**0.857*****	1.687	1.666	0.128	12.996	**0.000002**	**0.981*****	3.127	–1.230	0.032	–38.350	**0.000009**

****P* < 0.01; [W] weight

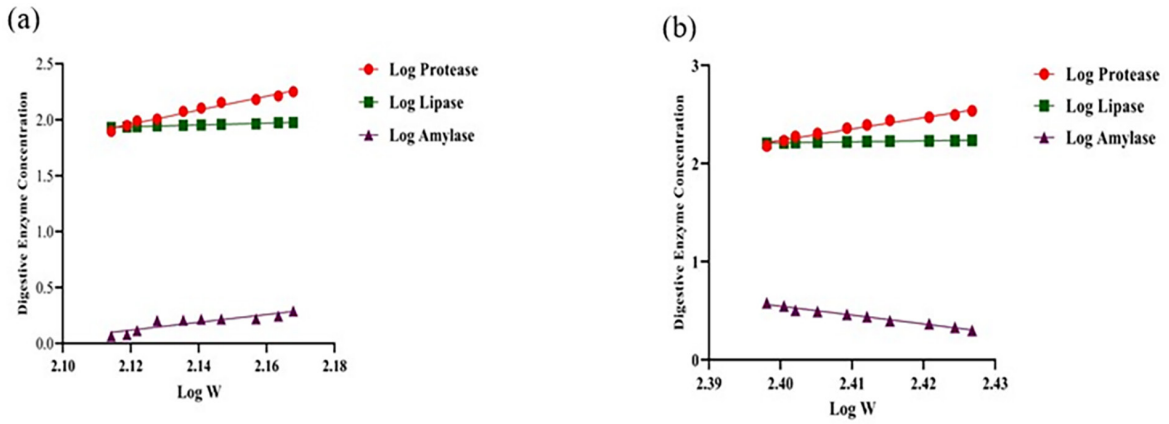

Figure 3. Correlation of log body weight (W) with digestive enzyme (protease, lipase, amylase) concentration (mean) in [A] juvenile and [b] adult group of *Notopterus notopterus*.

Table 4. Regression analysis of log relative gut length (RGL) and gut length (GL) correlation with digestive enzyme (protease, lipase, amylase) concentration in juvenile and adult group of *Notopterus notopterus*.

Relations	Group 1: juvenile						Group 2: adult					
	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 3	*P*-value	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 3	*P*-value
RGL (x) Log protease (y)	0.983***	0.033	0.275	0.006	40.321	**0.000002**	**0.945*****	0.173	0.279	0.012	22.059	**0.000003**
RGL (x) Log lipase (y)	**0.971*****	–3.482	2.094	0.067	30.926	**0.000003**	**0.937*****	–6.211	3.171	0.155	20.454	**0.000002**
RGL (x) Log amylase (y)	**0.872*****	0.528	0.420	0.030	13.845	**0.000004**	**0.909*****	0.992	–0.353	0.021	–16.763	**0.000003**
Log GL (x) Log protease (y)	**0.992*****	0.132	0.482	0.007	60.830	**0.000002**	**0.989*****	0.561	0.424	0.008	52.428	**0.000001**
Log GL (x) Log lipase (y)	**0.985*****	–6.044	3.678	0.085	43.107	**0.000003**	**0.982*****	–9.128	4.813	0.120	39.843	**0.000003**
Log GL (x) Log amylase (y)	**0.868*****	0.999	0.731	0.053	13.622	**0.000007**	**0.967*****	1.805	–0.540	0.018	–29.074	**0.000001**

****P* < 0.01; [RGL] relative gut length; [GL] gut length

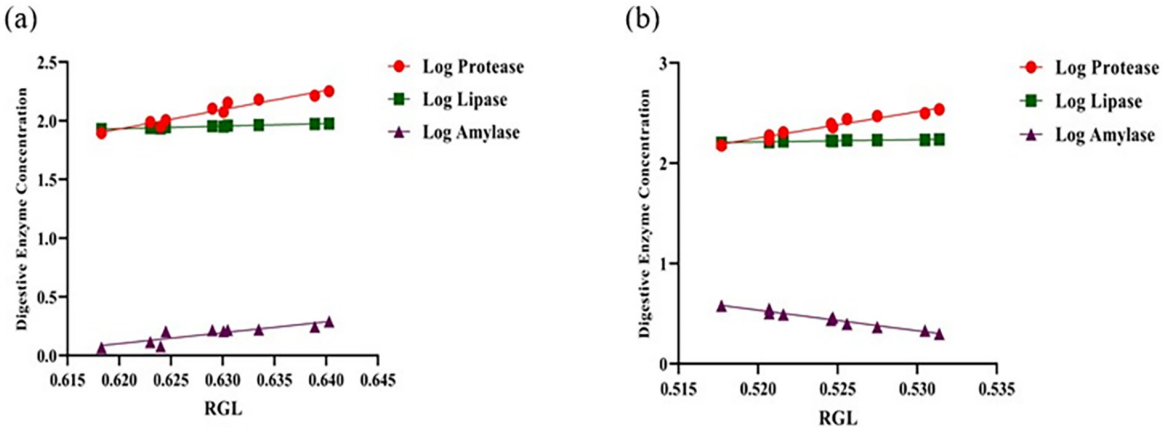

Figure 4. Correlation of relative gut length (RGL) with digestive enzyme (protease, lipase, amylase) concentration (mean) in [A] juvenile and [b] adult group of *Notopterus notopterus*.

Table 5. Regression analysis of condition factor (K) correlation with digestive enzyme (protease, lipase, amylase) concentration in juvenile and adult group of *Notopterus notopterus*.

Relations	Group 1: juvenile						Group 2: adult					
	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 0	*P*-value	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 0	*P*-value
Condition factor (x) Log protease n (y)	**0.983*****	–0.181	0.504	0.012	41.297	**0.000001**	**0.967*****	0.138	0.130	0.004	28.720	**0.000002**
Condition factor (x) Log lipase (y)	**0.990*****	–6.702	3.878	0.070	54.647	**0.000005**	**0.971*****	–2.851	1.484	0.047	31.116	**0.000002**
Condition factor (x) Log amylase (y)	**0.853*****	0.726	0.762	0.059	12.790	**0.000003**	**0.961*****	0.520	–0.167	0.006	–26.575	**0.000002**

***$P < 0.01$

Table 6. Regression analysis of Zihler's index (ZI) correlation with digestive enzyme (protease, lipase, amylase) concentration in juvenile and adult groups of *Notopterus notopterus*.

Relations	Group 1: juvenile						Group 2: adult					
	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 0	*P*-value	R (correlation coefficient)	a (intercept)	b (slope)	SE(b) (standard error)	t-value, when b = 0	*P*-value
Zihler's index (x) Log protease n (y)	**0.951*****	–309.826	172.605	7.401	23.319	**0.000002**	**0.971*****	–3088.58	1515.68	48.828	31.041	**0.000003**
Zihler's index (x) Log lipase (y)	**0.975*****	–2564.02	1338.796	39.927	33.530	**0.000003**	**0.959*****	–37597.6	17147.45	66.817	25.663	**0.000005**
Zihler's index (x) Log amylase (y)	**0.777*****	2.264	252.967	25.59	9.882	**0.000001**	**0.984*****	1370.962	–1964.96	46.351	–42.392	**0.000006**

***$P < 0.01$

Analysis of Digestive Enzyme Concentration in Adult Group of *Notopterus notopterus*

Analysis of TBL, W, GL, RGL, ZI, K, and protease, lipase, and amylase concentrations of the adult group are provided in Table 1.

TBL showed a significant ($P < 0.01$) positive correlation with protease and lipase concentration, whereas amylase concentration revealed a significant ($P < 0.01$) negative correlation with TBL in the adult group (Table *2)*. Figure 2b shows that protease and lipase concentrations increased with an increase in TBL, whereas amylase concentration decreased with an increase in TBL in the adult group of *N. notopterus*.

Total W exhibited a significant ($P < 0.01$) positive correlation with protease and lipase concentration, whereas amylase concentration revealed a significant ($P < 0.01$) negative correlation with the total W in the adult

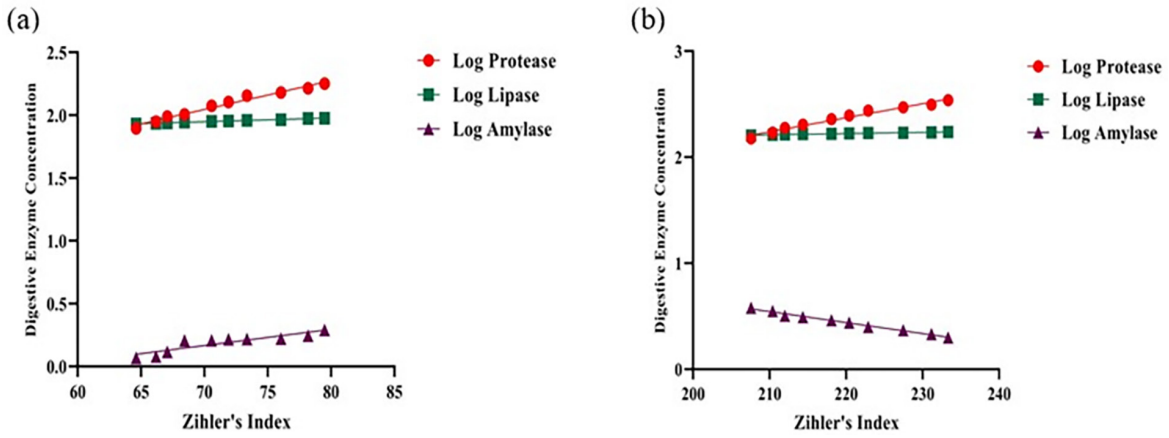

Figure 5. Correlation of Zihler's index (ZI) with digestive enzyme (protease, lipase, amylase) concentration (mean) in [A] juvenile and [A] adult group of *Notopterus notopterus*.

group (Table *3)*. Figure 3b indicated that protease and lipase concentrations increased with an increase in W, whereas amylase concentration decreased with an increase in W in the adult group.

RGL and GL showed a significant ($P < 0.01$) positive correlation with protease and lipase, whereas amylase concentration showed a significant ($P < 0.01$) negative correlation with RGL and GL in the adult group (Table 4). Figure 4b indicates that protease and lipase concentrations increased with an increase in RGL and GL, whereas amylase concentration decreased with an increase in RGL and GL in the adult group.

K relative to protease and lipase concentration was observed significantly ($P < 0.01$) positive, whereas amylase concentration exhibited a significantly ($P < 0.01$) negative correlation with K in the adult group (Table *5)*.

ZI showed a significant ($P < 0.01$) positive correlation with the protease and lipase concentrations, whereas the amylase concentration showed a significant ($P < 0.01$) negative correlation with ZI in the adult group (Table *6)*. Figure 5b indicates that protease and lipase concentrations increased with an increase in ZI, whereas amylase concentration decreased with an increase in ZI in the adult group.

DISCUSSION

The ecological niche of fish is not separated from their food (Bhuyan *et al*. 2022). The carnivorous fishes feed on aquatic weeds, prawns, nematodes, and small fishes (Kiran and Waghray 1998; Shillewar and Nanware 2009). Analysis of digestive enzyme concentration is an efficient technique to identify animal habit, mode of nutrition, and digestive physiology (Reiss *et al*. 2002).

Notopterus notopterus is a predatory fish. An individual with a body length of up to 27.5 cm is considered a juvenile, whereas one with a body length above 27.5 cm is considered an adult (Yanwirsal *et al*. 2017). In present study, digestive enzyme concentration (protease, lipase and amylase) was determined relative to TBL, W, GL, and RGL in juvenile and adult groups of *N. notopterus*. Moreover, ZI and K relative to digestive enzyme protease, lipase, and amylase concentrations were also evaluated.

N. notopterus is a carnivorous fish (Scharnweber *et al*. 2013; Keppler *et al*. 2015; Nandi and Saikia 2015). Figures 2a and b of present study showed an increasing pattern of amylase concentration relative to body length and GL at the juvenile stage, whereas a decreasing pattern of amylase concentration at the adult stage. This pattern of amylase concentration revealed that *N. notopterus* preferred to feed more on aquatic weed and phytoplankton at the juvenile stage, whereas it shifts to feeding on carbohydrates at the adult stage, as reported by Gogoi and Biswas (2015) and Gupta *et al*. (2021) in *N. notopterus* previously. In current study, we clearly determined the amylase concentration in the gut of *N. notopterus*, as Chakrabarti *et al*. (2006) reported previously in carnivorous fishes.

GL and RGL in *N. notopterus* showed significant positive correlation with total length and weight relative to protease and lipase concentration in the present study, as Riaz and Naeem (2020) and Lal *et al*. (2022) reported previously in carnivorous fish. In the present study, it was observed that GL increases with increasing total length of *N. notopterus*, as reported by Riaz and Naeem (2020) that the carnivorous fish GL increases as they grow in size.

N. notopterus W showed significant positive correlation with protease and lipase concentration in the present study (Figures 3a and b), as reported by Riaz and Naeem (2020) and Lal *et al*. (2022), whereas Hofer and Schiemer (1981)

reported that W showed significant negative correlation with protease and lipase concentrations in carnivorous fish.

In the present study, we observed high concentration of protease in gut of *N. notopterus*, as it was reported by Hidalgo *et al*. (1999) and Lundstedt *et al*. (2004) in the gut of *Oncorhynchus mykiss* and *Pseudoplatystoma corruscans*. Carnivorous fishes have relatively high protease and lipase concentrations as compared to herbivorous fishes (Drewe *et al*. 2004; Chakrabarti *et al*. 2006).

Figure 3b of present study indicated that amylase concentration exhibited significant negative correlation with W in adult *N. notopterus* species, as reported by Hofer and Schiemer (1981), whereas Riaz and Naeem (2020) and Lal *et al*. (2022) reported amylase significant positive correlation with W of carnivorous fishes.

The RGL of *N. notopterus* was observed to be between 0.55–0.88 in this present study, in comparison with what Lal *et al*. (2022) reported in *Terapon jarbua* at 1.23–1.99 previously. Figures 4a and b indicated that protease concentration increases with increasing TBL, GL, and RGL in the juvenile and adult groups of *N. notopterus* in the present study, as reported by Lundstedt *et al*. (2004) previously.

In present study, we also found that lipase concentration varies with increase in TBL, GL, and RGL of fish in both juvenile and adult groups, as reported by Tengjaroenkul *et al*. (2000). In present study, lipase concentration in *N. notopterus* showed significant positive correlation with GL, whereas Lal *et al*. (2022) reported negative significant correlation with GL of *Terapon jarbua*.

In the present study, the ZI values (Table 1) of *N. notopterus* were found to be between 23.35–85.36 (juvenile) and 258.63–782.64 (adult), in comparison with those evaluated by Lal *et al*. (2022) in *Terapon jarbua* at 0.32–0.92 previously. Figures 5a and b of present study showed that protease and lipase concentrations increased with increasing ZI, as reported by Riaz and Naeem (2020) in *Wallago attu* previously.

Figures 5a and b of present study showed that ZI positively correlated with the amylase concentration in the juvenile group, as reported by Riaz and Naeem (2020), and Lal *et al*. (2022) in *Wallago attu*. In the adult group of *N. notopterus*, amylase showed a significant negative correlation with ZI in the present study, whereas Riaz and Naeem (2020) reported that ZI exhibited a positive significant correlation with amylase in *Wallago attu*.

Table 5 of present study revealed that K exhibited significant positive correlation with protease and lipase concentration of *N. notopterus*, as previously reported by Riaz and Naeem (2020) in *Wallago attu*. Amylase concentration showed a negative correlation with K in adult group of *N. notopterus* (Table 5) in the present study, whereas Riaz and Naeem (2020) reported amylase concentration exhibited a significant positive correlation with K in *Wallago attu*.

The high concentration of protease and low concentration of amylase in juvenile and adult groups of *N. notopterus* evidently provides the carnivorous feeding habit of *N. notopterus*, as Riaz and Naeem (2020), Lal *et al*. (2022), Hidalgo *et al*. (1999), and Furne *et al*. (2005) reported previously. Moreover, Deguara *et al*. (2003), Lundstedt *et al*. (2004), and Natalia *et al*. (2004) reported that carnivorous fishes at adult stage eat more protein rich feed and fewer amounts of carbohydrates.

CONCLUSION

To the best of our knowledge, this study is first attempt to compare gut digestive enzyme (protease, lipase, and amylase) concentration relative to TBL, W, GL, RGL, ZI, and K in juvenile and adult groups of wild *Notopterus notopterus* fish. The high protease and lipase concentrations and low amylase concentration in both juvenile and adult groups of species can be linked to their carnivorous feeding habits. The significant variation was found in RGL of both juvenile and adult groups of this species. The present study also suggests that variation in RGL of all age groups of this wild fish may be due to influence of environment. The results of present study can be useful in understanding the feeding habit and digestive physiology of carnivorous fish species. Moreover, these results can be used to manage this species in the wild to fill the gap of knowledge in this area.

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STATEMENT ON CONFLICT OF INTEREST

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