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Statistical Approach on Biochemical Analysis of *Barilius bendelisis*

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ABSTRACT
To determine the nutritive value, a proximate analysis on the biochemical contents of a commonly consumed and available popular freshwater small indigenous fish (SIS) Barilius bendelisis of Torsa, Raidak-1, Jaldhaka(Mansai) rivers of Cooch Behar district of West Bengal, India was conducted. The most significant nutrient content of Barilius bendelisis (locally known as Boroli fish) was calculated using standard 'AOAC' protocols, such as protein, fat, moisture, and ash (AOAC, 2003; AOAC, 2005; AOAC, 1990). The crude protein level ranged between 16.86 and 16.72 percent, with the highest protein content observed in fish collected in Jaldhaka (Mansai) and the lowest in the Raidak-1 river. The fat content ranged between 6.54 and 6.45 percent. The moisture level ranged from 73.38 percent to 72.99 percent, whereas the ash content varied between 3.42 percent and 3.34 percent. The current work clearly demonstrates that 'SIS' is an economical source of quality protein that can provide nutritional security, financial security, and can meet the locality's protein needs. Enhancement of aquaculture output and consumption of small fishes is advocated as a better solution to address this region's protein demand.Regression analysis was also performed between the factors to determine their effects that yielded: $y = 1/(1+ \exp(-(a + b_1x_1 - b_2x_2 - b_3x_3)))$; where y represents the CPUE of Barilius bendelisis, x_1 represents moisture content, x_2 represents protein content and x_3 denotes the amount of crude fat. The correlation between the proximate variables revealed that, the value of 'r' was maximum between Moisture and Crude fat (r=0.76 and 0.39) at 0.01 level (2 tailed).
Keywords: *Small indigenous species (SIS), Proximate analysis, Protein hunger, Regression Analysis, Nutritive value.*

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INTRODUCTION

The overall population is currently around seven billion, with a projected increase to nine billion by 2050 [1]. According to one report, the urgent demand for mass food production would increase by more than 40% by 2030 and more than 70% by 2050 [2]. Small indigenous fish species (SIS) can be an excellent choice for avoiding micronutrient deficiencies and associated disorders [4-5]. Fish, as the least expensive source of easily digestible animal protein which can take a significant role to meet protein hunger, employment, and nutritional security in the coming days [6].

Various freshwater fish species inhabit India, with over 2,246 indigenous finfish species identified to date [7]. Freshwater is home to approximately 765 species, with 450 of those species classified as tiny indigenous fishes (SIS) which are a good supply of micronutrients and a cheap source of high-quality animal protein [8]. 'SIS' are species that can grow to a maximum length of 25-30 cm during the development or adult phase of their lifecycle [9].

Data on biochemical constituents will make it easier for a processing technologist to define the ideal preparation and capacity conditions with the goal of protecting quality to the greatest extent possible, as fish is a well transitory product and quality deterioration is caused by changes in the proximate composition. Understanding the biochemical elements of fish has become critical for nutritionists and dieticians.

Due to a lack of acceptable data on the biochemical composition of fish from rivers in West Bengal's northern region, consumers and fisheries workers are left with insufficient information on the importance of a few fish species in their daily diets [10]. The purpose of this study is to provide significant information on the biochemical makeup of a popular indigenous small fish called *Barilius bendelisis*

(locally known as boroli fish) found in Torsa, Raidak-1, Jaldhaka(Mansai) river in the Cooch Behar District of West Bengal, India.

MATERIAL AND METHODS

Sampling Site

Barilius bendelisis collected from fishermen landing at the bank of the Torsa, Raidak-1, Jaldhaka (Mansai) river of Cooch Behar district of West Bengal. It is necessary to document a comprehensive nutrient profile with a specific focus on popular small indigenous species (SIS) *Barilius bendelisis* in Cooch Behar for pre-monsoon, monsoon and the post-monsoon period during the period of March 2018 to November 2018 and March 2019 to November 2019 in different spots of each river (**Table 1**). The collected fish samples were transported in an insulated icebox with a proper identification mark to the laboratory.

Table-1 GPS location of sampling spots of *Barilius bendelisis*

River	Spot of fish collection	GPS Readings with height from sea level
Torsa river	ST1	26°21'27"N, 89°22'42"E, 158 ft
	ST2	26°17'13"N , 89°27'33"E, 122 ft
	ST3	26°15'5"N , 89°36'37"E, 109 ft
Raidak-1 river	SR1	26°21'07''N, 89°40'29''E, 122ft
	SR2	26°18'39''N , 89°40'14''E,118 ft
	SR3	26°13'09''N , 89°41'37''E,104 ft
Jaldhaka (Mansai river)	SJ1	26°21'35"N, 89°13'31"E, 146ft
	SJ2	26°19'10"N , 89°14'23"E, 157ft
	SJ3	26°15'34"N, 89°15'59"E, 137ft

Preparation of samples

In the laboratory, the fish samples identified using the taxonomy keys, the color, color patterns, spots, and their picture taken by a DSLR camera. The fishes were identified by studying their morphometric and meristic characters and by taking into consideration the literature of Talwar and Jhingran, 1991 and Jayaram, 1999 and 2006 [11-13]. The notable sample was then set apart in triplicates as Standard length, absolute length, and weight of fish sample recorded. The muscle for selected fishes was separated from their body just after washing. A sharp scalpel of stainless steel was used for dressing the fish species due to the smaller size of the species. The samples were dressed, beheaded, deskinned, and filtered as per manual followed by thorough washing with distilled water. The fillet of the fish samples was minced, homogenized, packaged, labeled, and stored frozen until analysis. Fishes were thawed and removal of the bone and skin from the flesh to analyzed by standard 'AOAC' procedures (**AOAC, 2003; AOAC, 2005; AOAC, 1990**) [14-16].

Total moisture content determination Procedure

Total moisture content was calculated by the standard procedure (AOAC, 2005). Triplicates of the pre-weigh samples were put into Petri dishes dried in an air-dry spell oven. The ground fish sample were taken in a clean dry petri dish and kept in an oven at 105ºC for 2 hours. After cooling in a desiccator and weighed (w_1). About 10 gm portion of the sample (w_2) was taken in the preweighed petri dish and then kept in the oven at 105˚C overnight. The dish was cooled in a desiccator and weighed again (w_3). Once again the petri dish was kept in the oven for half an hour and cooled as before and finally weighed to obtain the reproducible weights.

Calculation:

Moisture content (%) = $(w_2 - w_3) / (w_2 - w_1) \times 100$

Total Crude Protein Determination

Determination of crude protein was done with the method prescribed by AOAC, 2005. Principle: By boiling with concentrated sulfuric acid, the nitrogenous compounds in the sample are converted into ammonium sulfate. Upon distillation with excess alkali, the ammonia is liberated and the volume is estimated by titration with standardized sulfuric acid. Procedure: Exactly 0.1-0.2 g of wet sample was weighed into a Kjeldahl flask. A pinch of digestion mixture ($CuSO_4$ and K_2SO_4 were mixed in the ratio 1:8 and finely powdered) and 10 ml of concentrated H_2SO_4 was added. It was then heated (digested over a sand bath) slowly till the solution starts boiling first and then vigorously until the solution turns into colorless. The sample was cooled. The sample made up to the desired volume (100ml). A conical flask containing 10 ml of boric acid with a few drops of boric acid indicator (pink in color) was placed at the receiving end of the distillation apparatus in such a way that the tip of the condenser is to some extent immersed in boric acid. 5ml of the made-up sample was pipetted out into the distillation apparatus. 10ml of 40% NaOH as shown excess by phenolphthalein indicator was added to the distillation unit followed by rinsing with little distilled water. The unit was made air-tight. The content was steam distilled for 5 minutes. The color of the solution turns green. The condenser tip was washed with a small amount of

water after lowering the flask. The green solution in the receiving flask is green at this stage. The content was titrated against N/100 H_2SO_4 until the original pink color was restored. The volume of acid used for titration was noted for calculation. The distillation and titration process was repeated to get an accurate value.

Calculation:

1000 ml 1N H_2SO_4 =14g N_2

1ml 1 N H_2SO_4 = 0.014g N_2

1ml 0.01 N/100 H_2SO_4= 0.00014g nitrogen or (0.14/1000)

Protein content (%) = (Sample Titre Volume - Blank Titre Volume) x 1.4007 Normality/Wt. of Sample X Protein factor

Total protein was considered by multiplying the measured nitrogen by a protein conversion factor of 6.25.

Protein (%) = Nitrogen (%) × 6.25

Total Crude Fat Determination

Crude fat was calculated by the method given by AOAC, 1990.

10 gm of the dried fish sample (w_1) was placed in a thumble placed in a soxhlet apparatus and approximately 200ml ether was added and distilled for 16 hrs. After cooling the apparatus, the solvent was filtered into a pre-weighed conical flask (w_2). The ether was then removed by evaporation and the flask with lipid was dried at 80-100 ºC, cooled in a desiccator, and weighed (W_3).

Calculation: The fat content was then calculated using the formula Fat content (g/100g) = (w_3-w_2)/ w_1 x 100

Total Ash Determination

Ash content was calculated by the procedure prescribed in AOAC, 2003

Silica crucible was heated to 600ºC in a muffle furnace for one hour, cooled in a desiccator, and weighed (w_1). 2g of dried material was accurately weighed into a crucible and burned on a low flame while resting on a clay triangle to burn the organic matter (w_2). The burned material was then placed in the previously preheated (600° C) muffle furnace and burned for 6-8 hours, yielding grayish-white ash. The crucible was weighed after cooling in a desiccator (w_3). The crucible was heated again for further 30 mins to confirm completion of ashing, cooled, and weighed again.

Calculation:

Ash content (g/100g) = w_3 -w_1/ w_2 -w_1 X 100 Where, w_1= Weight of crucible, w_2 = Dry substance and crucible weight w_3 = Weight of crucible after ashing.

Statistical Analysis

The statistical investigation was done utilizing SPSS 21, PAST 4.03, MS Excel 2007 software.

Ethical issues

The examination was as per the Declaration of Helsinki and guidelines on good clinical practice locally accessible. It was likewise endorsed by the institutional ethics board and morals committee [17].

RESULTS

The proximate composition of *Barilius bendelisis* are shown (**Table 2-4**) for Torsa, Raidak-1, Mansai(Jaldhaka) river for three periods at three sites of each river respectively. The outcomes from the present examination uncovered that moisture, protein, fat, and ash showed variations in three seasons at three sites for the collected small fish species.

Table-2 Proximate analysis of *Barilius bendelisis* collected from three sampling spots of Torsa river

River	Site	Period	Moisture(g/100g)	Crude Fat(g/100g)	Crude Protein(g/100g)	Ash(g/100g)
Torsa	ST1	Pre monsoon	72.77	6.38	16.66	3.42
	ST2	Pre monsoon	72.2	6.37	16.54	3.37
	ST3	Pre monsoon	72.38	6.4	16.88	3.46
Mean Value(Mean ±2sd)			72.45 ±0.33	6.38 ±0.0173	16.69 ±0.19	3.42 ±0.05
Torsa	ST1	Monsoon	72.45	6.31	16.6	3.41
	ST2	Monsoon	72.39	6.3	16.52	3.33
	ST3	Monsoon	72.11	6.33	16.65	3.4
Mean Value(Mean ±2sd)			72.32 ±0.20	6.31 ±0.02	16.59 ±0.07	3.38 ±0.05
Torsa	ST1	Post monsoon	73.58	6.55	16.98	3.48
	ST2	Post monsoon	74.69	6.45	16.58	3.42
	ST3	Post monsoon	74.71	6.98	17.28	3.49
		min	73.58	6.45	16.58	3.42
		max	74.71	6.98	17.28	3.49
Mean Value(Mean ±2sd)			74.33 ±0.73	6.66 ±0.32	16.95 ±0.39	3.46 ±0.04
		Approx. 95% of the data lies between ± 2 SD				

Table-3 Proximate analysis of *Barilius bendelisis* collected from three sampling spots of Raidak-1 river

River	Site	Period	Moisture(g/100g)	Crude Fat(g/100g)	Crude Protein(g/100g)	Ash(g/100g)
Raidak-1	SR1	Pre monsoon	72.19	6.48	16.76	3.32
	SR2	Pre monsoon	72.15	6.5	16.68	3.35
	SR3	Pre monsoon	72.4	6.52	16.88	3.38
Mean Value(Mean ±2sd)			72.25 ±0.15	6.5 ±0.02	16.77 ±0.12	3.35 ±0.03
Raidak-1	SR1	Monsoon	72.38	6.34	16.6	3.4
	SR2	Monsoon	72.22	6.33	16.55	3.33
	SR3	Monsoon	72.16	6.39	16.67	3.47
Mean Value(Mean ±2sd)			72.25 ±0.13	6.35 ±0.03	16.61 ±0.06	3.4 ±0.07
Raidak-1	SR1	Post monsoon	74.48	6.52	16.77	3.45
	SR2	Post monsoon	74.49	6.5	16.62	3.4
	SR3	Post monsoon	74.4	6.77	16.98	3.48
Mean Value(Mean ±2sd)			74.46 ±0.05	6.59 ±0.17	16.79 ±0.20	3.44 ±0.04
		Approx. 95% of the data lies between ± 2 SD				

Table-4 Proximate analysis of *Barilius bendelisis* collected from three sampling spots of Jaldhaka (Mansai)river

River	Site	Period	Moisture(g/100g)	Crude Fat(g/100g)	Crude Protein(g/100g)	Ash(g/100g)
Jaldhaka	SJ1	Pre monsoon	73.98	6.59	17.01	3.4
	SJ2	Pre monsoon	73.13	6.31	16.98	3.33
	SJ3	Pre monsoon	74.14	6.66	17.08	3.44
Mean Value(Mean ±2sd)			73.75 ±0.6	6.52 ±0.21	17.02±0.05	3.39 ±0.06
Jaldhaka	SJ1	Monsoon	72.3	6.42	16.59	3.32
	SJ2	Monsoon	72.28	6.4	16.22	3.3
	SJ3	Monsoon	72.33	6.49	16.7	3.38
Mean Value(Mean ±2sd)			72.30 ±0.02	6.43 ±0.05	16.50 ±0.28	3.33 ±0.04
Jaldhaka	SJ1	Post monsoon	74.44	6.68	17.1	3.31
	SJ2	Post monsoon	73.26	6.52	16.98	3.3
	SJ3	Post monsoon	74.56	6.78	17.12	3.35
Mean Value(Mean ±2sd)			74.09 ±0.81	6.66 ±0.15	17.07 ±0.08	3.32 ±0.02
				Approx. 95% of the data lies between ± 2 SD		

Seasonal variation of proximate analysis of *Barilius bendelisis* at different sites in Torsa River. Three sampling spots in the river Torsa namely Kawalipara, Harinchawra, and Balarampur were selected for the collection of *Barilius bendelisis*. Proximate analyses were studied in river Torsa at three seasons namely pre-monsoon, monsoon, and post-monsoon period for two consecutive years. The proximate analysis of Barilius bendelisis recorded in the premonsoon period at ST1 spot showed the range Moisture (72.75 g/100g - 72.77 g/100g), Crude Fat (6.37 g/100g -6.38 g/100g), Crude protein (16.65 g/100g -16.67 g/100g), Ash (3.40 g/100g- 3.43 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at ST1 spot showed the range Moisture (72.43 g/100g -72.45 g/100g), Crude Fat (6.30 g/100g- 6.32 g/100g), Crude protein (16.58 g/100g -16.66 g/100g), Ash (3.40 g/100g- 3.42 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at ST1 spot showed the range of Moisture (73.57 g/100g - 73.58 g/100g), Crude Fat (6.54 g/100g- 6.56 g/100g), Crude protein (16.97 g/100g -16.98 g/100g), Ash (3.47 g/100g- 3.48 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at ST2 spot showed the range of Moisture (72.20 g/100g- 72.21 g/100g), Crude Fat (6.36 g/100g -6.37 g/100g), Crude protein (16.52 g/100g -16.54 g/100g), Ash (3.36 g/100g- 3.38 g/100g). The proximate analysis of Barilius bendelisis recorded in the monsoon period at ST2 spot showed the range of Moisture (72.38 g/100g - 72.39 g/100g), Crude Fat (6.30 g/100g- 6.31 g/100g), Crude protein (16.51 g/100g -16.53 g/100g), Ash (3.31 g/100g- 3.34 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at ST2 spot showed the range of Moisture (74.67 g/100g -74.69 g/100g), Crude Fat (6.44 g/100g - 6.45 g/100g), Crude protein (16.57 g/100g -16.59 g/100g), Ash (3.41 g/100g- 3.42 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at ST3 spot showed the range of Moisture (72.37 g/100g - 72.39 g/100g), Crude Fat (6.39 g/100g – 6.40 g/100g), Crude protein (16.87 g/100g -16.88 g/100g), Ash (3.46 g/100g- 3.57 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at ST3 spot showed the range of Moisture (72 g/100g – 72.11 g/100g), Crude Fat (6.30 g/100g – 6.33 g/100g), Crude protein (16.55 g/100g -16.65 g/100g), Ash (3.38 g/100g- 3.40 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at ST3 spot showed the range of Moisture (74.41 g/100g -74.71 g/100g), Crude Fat (6.96g/100g- 6.98 g/100g), Crude protein (17.27 g/100g -17.28 g/100g), Ash (3.47 g/100g- 3.49 g/100g). Seasonal variation of proximate analysis of

Barilius bendelisis at different sites in Raidak-1 River. Three sampling spots in the river Raidak-1 namely Bansraja, Ranirhut, and Kerochhar (Sarkar para) were selected for the collection of *Barilius bendelisis*. Proximate analyses were studied in river Raidak-1 at three seasons namely pre-monsoon, monsoon, and post-monsoon period for two consecutive years. The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SR1 spot showed the range of Moisture (72.17 g/100g - 72.19 g/100g), Crude Fat (6.46 g/100g – 6.48 g/100g), Crude protein (16.74 g/100g -17.77 g/100g), Ash (3.30 g/100g- 3.32 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at SR1 spot showed the range of Moisture (72.36 g/100g - 72.38 g/100g), Crude Fat (6.33 g/100g- 6.34 g/100g), Crude protein (16.5 g/100g -16.61 g/100g), Ash (3.40 g/100g- 3.42 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SR1 spot showed the range of Moisture (74.46 g/100g - 74.48 g/100g), Crude Fat (6.51 g/100g – 6.52 g/100g), Crude protein (16.76 g/100g -16.77 g/100g), Ash (3.43 g/100g- 3.45 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SR2 spot showed the range of Moisture (72.13 g/100g - 72.15 g/100g), Crude Fat (6.5 g/100g – 6.52 g/100g), Crude protein (16.67 g/100g -16.68 g/100g), Ash (3.34 g/100g- 3.35 g/100g). The proximate analysis of Barilius bendelisis recorded in the monsoon period at SR2 spot showed the range of Moisture (72.20 g/100g - 72.22 g/100g), Crude Fat (6.3 g/100g – 6.34 g/100g), Crude protein (16.53 g/100g - 16.55 g/100g), Ash (3.30 g/100g- 3.34 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SR2 spot showed the range of Moisture (74.48 g/100g - 74.50 g/100g), Crude Fat (6.5 g/100g – 6.51 g/100g), Crude protein (16.6 g/100g - 16.63 g/100g), Ash (3.40 g/100g- 3.41 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SR3spot showed the range of Moisture (72.38 g/100g - 72.41 g/100g), Crude Fat (6.5 g/100g – 6.52 g/100g), Crude protein (16.87 g/100g -16.88 g/100g), Ash (3.28 g/100g- 3.38 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at SR3spot showed the range of Moisture (72.15 g/100g - 72.16 g/100g), Crude Fat (6.37 g/100g – 6.39 g/100g), Crude protein (16.64 g/100g -16.67 g/100g), Ash (3.46 g/100g- 3.48 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SR3spot showed the range of Moisture (74.4 g/100g - 74.41 g/100g), Crude Fat (6.74 g/100g - 6.77 g/100g), Crude protein (16.96 g/100g -16.98 g/100g), Ash (3.47 g/100g- 3.48 g/100g). Seasonal variation of proximate analysis of *Barilius bendelisis* at different sites in Mansai (Jaldhaka) River. Three sampling spots in the river Mansai (Jaldhaka) namely Angerkata Khaterbari, Tekonia, and Shibpur were selected for the collection of *Barilius bendelisis*. Proximate analyses were studied in the river Mansai (Jaldhaka) at three seasons namely pre-monsoon, monsoon, and post-monsoon period for two consecutive years. The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SJ1 spot showed the range of Moisture (73.97 g/100g – 73.98 g/100g), Crude Fat (6.58 g/100g – 6.59 g/100g), Crude protein (17g/100g -17.02 g/100g), Ash (3.38 g/100g- 3.40 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at SJ1 spot showed the range of Moisture (72.26 g/100g - 72.31 g/100g), Crude Fat (6.38 g/100g – 6.42 g/100g), Crude protein (16.96 g/100g -17.01 g/100g), Ash (3.30 g/100g- 3.32 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SJ1 spot showed the range of Moisture (74.42 g/100g – 74.44 g/100g), Crude Fat (6.67 g/100g -6.68 g/100g), Crude protein (17 g/100g -17.10 g/100g), Ash (3.10 g/100g- 3.31 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SJ2 spot showed the range of Moisture (72.12 g/100g - 73.13 g/100g), Crude Fat (6.30 g/100g- 6.38 g/100g), Crude protein (16.97 g/100g -16.98 g/100g), Ash (3.31 g/100g- 3.33 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at SJ2 spot showed the range of Moisture (72.26 g/100g - 72.28 g/100g), Crude Fat (6.38 g/100g – 6.41 g/100g), Crude protein (16.96 g/100g -16.98 g/100g), Ash (3.30 g/100g- 3.32 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SJ2 spot showed the range of Moisture (72.27 g/100g - 72.26 g/100g), Crude Fat (6.37 g/100g – 6.52 g/100g), Crude protein (16.96 g/100g -16.98 g/100g), Ash (3.30 g/100g- 3.32 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the pre-monsoon period at SJ3 spot showed the range of Moisture (74.12 g/100g – 74.14 g/100g), Crude Fat (6.64 g/100g – 6.67 g/100g), Crude protein (17.07 g/100g -17.08 g/100g), Ash (3.41 g/100g- 3.44 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the monsoon period at SJ3 spot showed the range of Moisture (72.30 g/100g - 72.34 g/100g), Crude Fat (6.47 g/100g – 6.49 g/100g), Crude protein (17.05 g/100g -17.08 g/100g), Ash (3.37 g/100g- 3.38 g/100g). The proximate analysis of *Barilius bendelisis* recorded in the post-monsoon period at SJ3 spot showed the range of Moisture (74.55 g/100g – 74.57 g/100g), Crude Fat (6.77 g/100g – 6.78 g/100g), Crude protein (17.10 g/100g -17.20 g/100g), Ash (3.33 g/100g- 3.35 g/100g).

Proximate compositions such as Moisture (g/100g), Protein (g/100g), and Crude fat (g/100g) content of *Barilius bendelisis* were measured in two consecutive years Proximate composition Correlation was

calculated for two consecutive years and represented below (**Table 5-6**). Correlation is significant at the 0.01 level (2-tailed).

Table 5: The correlation matrix for biochemical parameters based on data analysed in year 2018.

	Moisture	Crude Fat	Crude Protein
Crude Fat	**0.763****		
Crude Protein	**0.273****	**0.590****	
Ash	**0.339****	**0.270****	-0.107

** Correlation is significant at the 0.01 level (2-tailed)

Table 6: The correlation matrix for biochemical parameters (based on data analysed in year 2019).

	Moisture	Crude Fat	Crude Protein
Crude Fat	**0.249****		
Crude Protein	**0.398****	**0.279****	
Ash	**0.253****	0.003	-0.053

** Correlation is significant at the 0.01 level (2-tailed)

The correlation between the proximate variables revealed that the value of **'r'** was maximum between Moisture and Crude fat (r=0.76 and 0.39) at 0.01 level (2 tailed).

Regression analysis on proximate composition

Further, both the year's data (2018 and 2019) were also subjected to regression analysis concerning proximate analysis viz. Moisture, Crude Protein, Crude Fat, Ash, and abundance (CPUE) of *Barilius bendelisis*. (**Figure 2-3**).

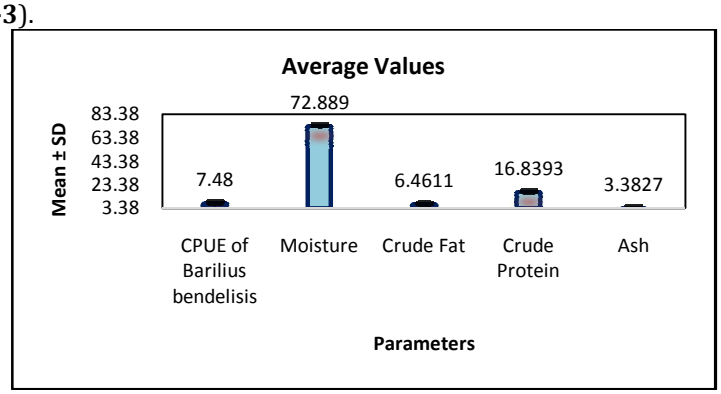

Figure 2: The values (mean ± S.E.) of moisture, crude fat, crude protein, ash, and CPUE of *Barilius bendelisis*: based on data collected in the year 2018

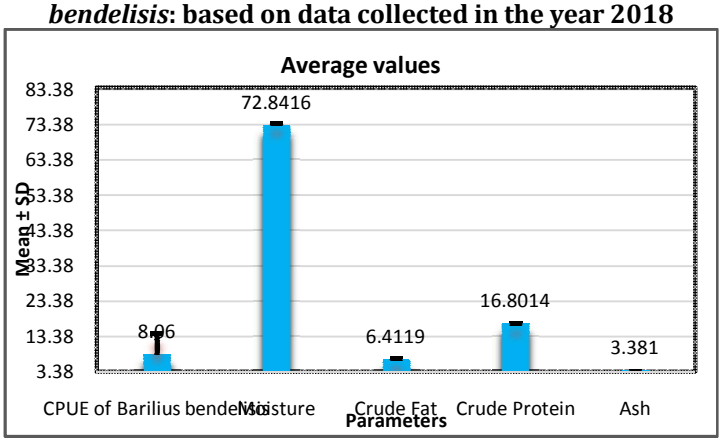

Figure 3: The values (mean ± S.E.) of moisture, crude fat, crude protein, ash and CPUE of *Barilius bendelisis*: based on data collected in year 2019

Model summary for the two consecutive years is represented **(Table 7)**. The regression model suggests R-value, R Square, adjusted R Square, Std. Error of the estimate and the value of Durbin-Watson were 0.757, 0.573, 0.562, 3.817, 0.621 respectively for the year 2018. Regression model showed R value, R Square, adjusted R Square, Std. Error of the estimate and the value of Durbin-Watson were 0.748, 0.559, 0.548, 4.110, and 0.602 respectively. R square values for both the years considered a moderate effect size. The value of Durbin-Watson is below 2, it generally indicates a positive autocorrelation.

Table 7: Model Summary Ash, Crude Protein, Moisture, Crude Fat as predictor variables and CPUE of *Barilius bendelisis* as dependent variable): Based on data collected in two consecutive years (2018 and 2019)

Model Summary[b]					
Year	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
2018	0.757[a]	0.573	0.562	3.817	0.621
2019	0.748[a]	0.559	0.548	4.110	0.602
a. Predictors: (Constant), Ash, Crude Protein, Moisture, Crude Fat					
b. Dependent Variable: CPUE of *Barilius bendelisis*					

For two consecutive years, F values calculated **52.388, 49.761** where CPUE of *Barilius bendelisis* is the dependent variable. Both the F values are statistically significant **(Table 8)**.

Table 8: Regression Analysis (F Value) on proximate analysis in two consecutive years

Model		Sum of Squares	df	Mean Square	F	Sig.
1 (2018)	Regression	3053.224	4	763.306	**52.388**	.000[b]
	Residual	2272.949	156	14.570		
	Total	5326.174	160			
1 (2019)	Regression	3362.363	4	840.591	**49.761**	.000[b]
	Residual	2652.137	157	16.893		
	Total	6014.500	161			
a. Dependent Variable: CPUE of *Barilius bendelisis*						

The degree of interrelation among the proximate composition and abundance (CPUE) of *Barilius bendelisis* is represented through, the Pearson correlation matrix **(Table 9-10)**.

Table 9: Correlation matrix for proximate composition (moisture, crude protein, crude fat, ash with CPUE of *Barilius bendelisis* (the year 2018).

The year 2018	CPUE of *Barilius bendelisis*	Moisture	Crude Fat	Crude Protein
Moisture	**0.642****			
Crude Fat	**0.687****	**0.762****		
Crude Protein	**0.448****	**0.273****	**0.590****	
Ash	**0.398****	**0.341****	**0.272****	-0.107

** Correlation is significant at the 0.01 level (2-tailed).

Table 10: Correlation matrix for proximate composition (moisture, crude protein, crude fat, ash with CPUE of *Barilius bendelisis* (the year 2019).

The year 2019	CPUE of *Barilius bendelisis*	Moisture	Crude Fat	Crude Protein
Moisture	**0.653****			
Crude Fat	**0.176****	**0.249****		
Crude Protein	**0.546****	**0.398****	**0.279****	
Ash	**0.287****	**0.253****	0.003	-0.053

**. Correlation is significant at the 0.01 level (2-tailed).

Collinearity Diagnostics

Collinearity Diagnostics were done in two consecutive years. For the two consecutive years, several eigenvalues are near zero, suggesting that the predictor variables are highly correlated **(Table 11)**.

Table 11: Collinearity Diagnostics (Biochemical parameter of *Barilius bendelisis*) based on two consecutive years (2018 and 2019)

Collinearity Diagnostics[a]							
Year	Eigenvalue	Condition Index	Variance Proportions				
			(Constant)	Moisture	Crude Fat	Crude Protein	Ash
2018	4.999	1.000	.00	.00	.00	.00	.00
	.000	116.958	.01	.00	.14	.04	.29
	.000	145.942	.05	.00	.16	.18	.15
	9.772E-05	226.187	.07	.26	.01	.30	.49
	2.765E-05	425.216	.86	.74	.69	.48	.07
2019	4.994	1.000	.00	.00	.00	.00	.00
	.005	30.992	.00	.00	.92	.00	.00
	.000	135.171	.01	.02	.03	.11	.77
	8.811E-05	238.077	.06	.97	.01	.28	.06
	6.056E-05	287.171	.93	.02	.04	.61	.17
a. Dependent Variable: CPUE of *Barilius bendelisis*							

Regression analysis was performed between the factors to determine their effects (Table) that yielded: $y = 1/(1+ \exp(-(a + b_1x_1 - b_2x_2 - b_3x_3)))$; where y represents the CPUE of *Barilius bendelisis*, x_1 represents moisture content, x_2 represents protein content and x_3 denotes the amount of crude fat.

DISCUSSION

The results obtained regarding the proximate analysis of *Barilius bendelisis* showed few similarities with the assessment made by other researchers in this field [18-20]. However, differences with other researchers may be accredited to different ecological/Physico-chemical conditions of different locations, periods, and ages of fish including duration during the time of sampling in the study period [21-22]. The protein content of fish mussels showed a range of 16.86% (g/100g) and 16.72% (g/100g) in our present study. The findings in our present study on a crude fat content range between 6.54% (g/100g) to 6.45% (g/100g) which is the moderate value of crude fat content have relevance with other reported data. The present study showed that the whole of fat and moisture for any of the studied fishes approximates 80% which has a close resemblance with other researchers in this field. In our study ash content of the studied fish ranges between 3.33% (g/100g) to 3.49% (g/100g). A larger amount of ash prompts having an extra elevated amount of mineral in bones. The present findings state that the low ash content might be due to a lesser amount of skeletons in small indigenous *Barilius bendelisis*. Similar values were observed in rivers of the North-Eastern Indian Himalayan region *Barilius bendelisis*[23-24]. The low Protein content of *Barilius bendelisis* collected from the most polluted sites of the river Fish collected from sites SJ2, SR2, ST2 of the River Mansai (Jaldhaka), Raidak-1, and Torsa river respectively which are adjacent to the town area showed lower protein contents. Similar results were found to study the impact of habitat degradation on proximate composition [25]. The decrease in protein contents in animals under toxic stress is due to the diversification of energy to accomplish impending energy demands. xxiv. The correlation between the proximate variables of *Barilius bendelisis* from three rivers. Three proximate compositions such as Moisture (g/100g), Protein (g/100g) and Crude fat (g/100g) content of *Barilius bendelisis* was measured.

CONCLUSION

Regression analysis was performed between the factors to determine their effects that yielded: $y = 1/(1+ \exp(-(a + b_1x_1 - b_2x_2 - b_3x_3)))$; where y represents the CPUE of *Barilius bendelisis*, x_1 represents moisture content, x_2 represents protein content and x_3 denotes the amount of crude fat. The correlation between the proximate variables revealed that, the value of 'r' was maximum between Moisture and Crude fat (r=0.76 and 0.39) at 0.01 level (2 tailed).

Taste, freshness, size, and other external aspects should not be the primary factors considered when deciding on a marketing and use strategy for fish. The findings of this study provided logical data and point-by-point learning of the proximate analysis of *Barilius bendelisis*, a significant selling fish in the Torsa, Raidak-1, Jaldhaka (Mansai) stream basin. Further detailed research are needed to investigate the amino acid profile, fatty acid profile, and mineral amounts in order to create a standard nutritional database of small indigenous fishes (SIS) from different rivers in this area. The current study considerably

expands the current information on the nutritional value of indigenous fish species accessible in West Bengal's Cooch Behar district. As a result, small indigenous fish *Barilius bendelisis* may be an useful option for people in rural and semi-urban areas to meet their daily dietary supplement requirements as well as combat micronutrient deficiencies in relation to their health advantages.

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CONFLICT OF INTEREST
The authors declare that they have no actual and potential conflict of interest.

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