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Preferred food items of Juvenile hilsa (*Tenualosa ilisha*) of the Meghna river estuary in Moju Chowdhury Hat, Lakshmipur

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Abstract
Monthly and size group-wise variation of feeding preference of juvenile hilsa was studied in the Meghna estuary, Lakshmipur from July to December, 2022. The specimens were dissected and gut contents were analysed by frequency of occurrence (FOC%), volumetric analysis (V%), relative gut length (RLG) and index of relative importance (IRI%) method. Fishes (n=120) were divided into 5 different size groups i.e. below 5 g, 5-10 g, 10-15 g, 15-20 g and 20-25 g. There were 26 genera of phytoplankton and 16 genera of zooplankton were recorded. According to FOC%, V% and IRI%, copepod and diatoms were found the major food constitutes in all the five size groups. Major bacillariophyceae genera were recorded *Coscinodiscus* sp., *Synedra* sp. and *Rhizosolenia* sp., and followed by copepoda (*Calanus* sp. and *Pseudodiaptomus* sp.). Based on the FOC% and V% we found sand particles as a constituent in the gut of hilsa (early age) was noticed but from IRI% value, it was observed that sand was only engulfed by hilsa with food particles during bottom feeding but not a preferred food item.

Keywords: Juvenile hilsa, gut analysis, preferred food, diatom, copepod

1. Introduction
The riverine environment in Bangladesh is one of nature's most beautiful treasures. Bangladesh boasts one of the largest and most active deltas in the world, with a 710-kilometer shoreline on the northern littoral of the Bay of Bengal fed by three major rivers: The Padma, the Meghna, and the Jamuna (Hussain and Mazid, 2001) [9]. The Meghna Estuary, located in the easternmost region of the Ganges Delta, transports the combined flow of the Ganges/Padma, Jamuna/Brahmaputra, and Meghna Rivers (Rashid, 2019) [15]. The Meghna river estuary in Bangladesh is the biggest estuarine environment and supports numerous fishing populations, as well as one of the most significant sites for the hilsa (*Tenualosa ilisha*) fishery (Rahman *et al*., 2012) [15]. Hilsa shad (*Tenualosa ilisha*) is a distinctive, commercial, migratory, and significant tropical fish, particularly in Bangladesh (Hasan *et al*. 2016) [7].
Food is any material of plant or animal origin that is ingested by organisms and provides them with nutritional sustenance (Begum *et al*., 2008) [3]. There is a significant link between fish and their preferred foods, which is also associated to increased fish output (Stevens, 2004) [18]. The food and feeding habits of hilsa (*Tenualosa ilisha*) have long been a study area of interest to Indo-Pacific fishery specialists. In Bangladesh, hilsa mostly move via the greatest body of water, the Padma-Meghna river system, for breeding and feeding (Hynes, 1950) [6]. However, there have been many studies on the food and feeding habits of hilsa throughout their entire life cycle, but a little study has been published that only focus on the food and feeding habits of juvenile hilsa along with adult hilsa. So, this present study is chosen mainly to focus on juvenile hilsa to determine their preferable food items and size group-wise variation in feeding habits.

2. Materials and Methods
2.1 Study area and period
Present study was driven on food and feeding habits of juvenile hilsa (*Tenualosa ilisha*) in Meghna River estuary, Moju Chowdhury Hat, Lakshmipur.

Moju Chowdhury Hat is a market village and tourist centre in Char Ramani Mohan Union of Lakshmipur Sadar Upazilla in Southeastern Bangladesh. The study area (Moju Chowdhury Hat) is located at 22.875° N latitude and 90.785° E longitude (Figure 1). The current study lasted from July 2022 to December 2022.

2.2 Fish Sample collection and preservation

Fish samples were collected at random from the Meghna River at Moju Chowdhury Hat in the Lakshmipur District. From July to December 2022, the fish were sampled once a month. The freshly caught specimens were kept in an ice box and transported to the laboratory of Noakhali Science and Technology University, Noakhali. The fish specimens were classified based on their size. Each month, 20 juvenile hilsa were chosen for gut content analysis from a total of 120 specimens with size ranged 1 g to 20 g. Fish were divided into four size groups based on their body weight: 1-5 g, 5-10 g, 10-15 g, and 15-20 g.

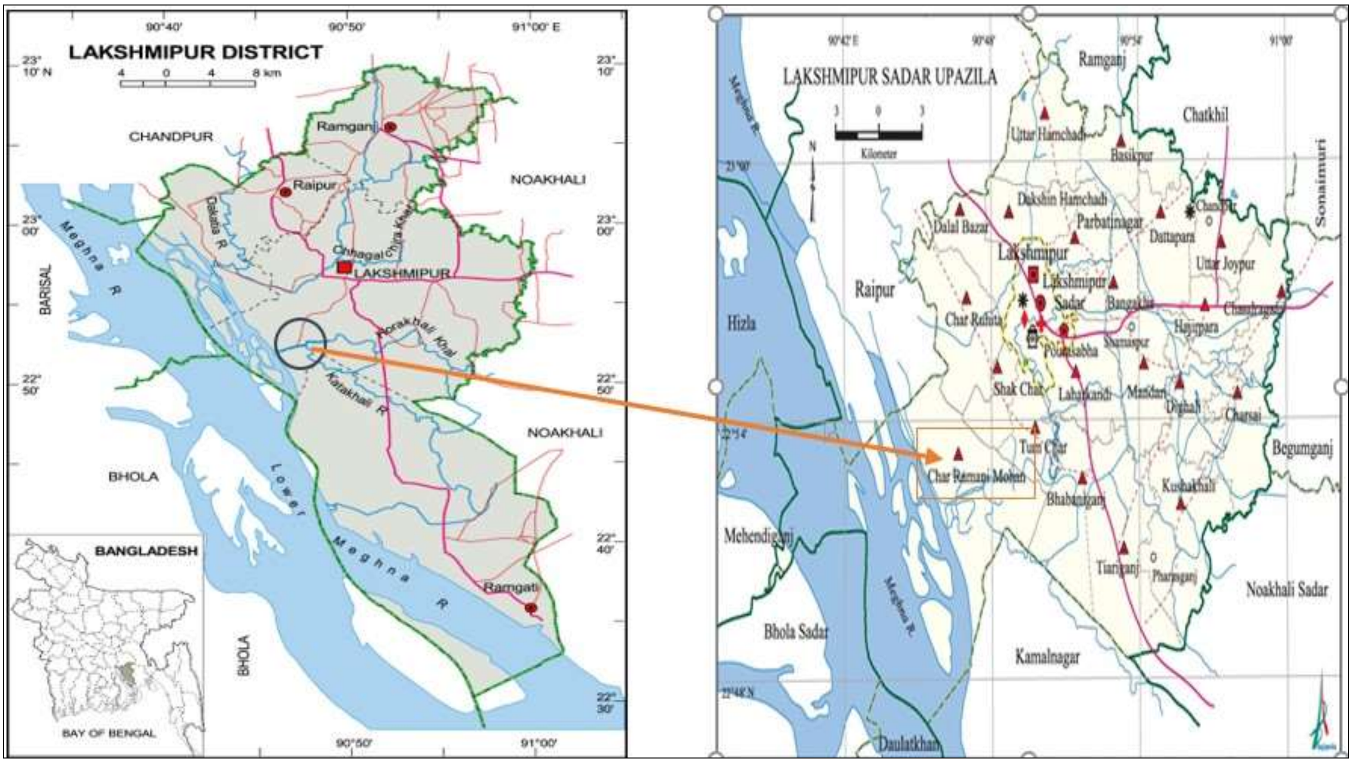

Fig 1: Map showing the present study area (Moju Chowdhury Hat) source: Bangla pedia

Total body length (cm), standard length (cm), body weight (g) were measured for all the hilsa fish sample. The total gut length (cm) of the fish was also measured after dissection.

2.3 Stomach collection

The fish were washed in running water and soaked in tissue paper. Fish were measured for standard length (SL) and body weight (BW) in each case. Their length ranged from 4 to 22 cm and their weight ranged from 1 to 20 grams. Individual fish stomachs were carefully dissected out into a clean petri dish and preserved with 10% formalin in a labelled small glass vial until analysis.

2.4 Gut content analysis

The contents of each stomach were examined separately. In the laboratory of the Department of Fisheries and Marine Science, NSTU, the gut contents of each fish were examined under a luminous stereoscopic microscope using a Sedgewick-Rafter counting cell for qualitative and quantitative analysis. The qualitative analysis of food items was carried out by comparing the planktons to available camera photographs and literature, and food items were identified up to the generic level. Plankton were identified to the genus level following the determination keys of Yamaji's (1984) [19] and Sahu *et al*. (2013) [16]. The frequency of occurrence method (Hynes, 1950; Hyslop, 1980) [6] [10], volumetric analysis method (Hynes, 1950) [6] and index of relative importance (Pinkas, 1971) [12] were used to estimate the gut contents quantitatively.

2.5 The Relative Gut Length

Relative gut length (RGL) is used for the classification of different sized fish as a carnivore, herbivore and omnivore as a main morphological variable. Generally, the relative gut length of fish species increases with the size and complexity of their food items. The RGL of the fish was determined by using the formula (Pinkas, 1971) [12];

$$RLG = \frac{\text{Total Gut Length}}{\text{Total Length}}$$

2.6 Frequency of occurrence (%)

In frequency of occurrence, the number of stomachs containing a specific type of food, expressed as a percentage of the total number of non-empty stomachs examined. It is the total number of food items present in the gut, as determined by the formula (Hynes, 1950; Hyslop, 1980) [6, 10].

$$F_i = \frac{100 n_i}{N}$$

Where: F_i, n_i and N represents frequency of occurrence (%) of the i food item in the sample, number of stomachs in which the i item is found and total number of stomachs with food in the sample respectively.

2.7 Volumetric (Point) analysis index (%): Hynes (1950) [6]

This technique is a variation on the eye estimation technique. Instead of assessing the volume directly by sight. Each food item in the stomach is assigned a certain number of points based on its volume in this method. The diet component with the greatest volume received 16 points. Every other component received 8, 4, 2, 1, or 0 points based on its volume in relation to the component with the highest volume. The percentage volumes of each sub sample were calculated as follows:

$$\propto = \frac{\text{Number of points allocated to component} \propto}{\text{Total points allocated to sub sample}} \times 100$$

Where, α is the percentage volume of the prey (food item) component α.

2.8 Index of Relative Importance

This index is calculated by adding the numerical and volumetric percentage values and multiplying by the percentage value of frequency of occurrence (Pinkas, 1971) [12]. As follows:

$$\text{Index of relative importance, iIRI} = (\%N_i + \%V_i) * O_i$$

Where, N_i, V_i and O_i represent percentages of number, volume and frequency of occurrence prey i respectively.

3. Results and Discussions

3.1 Identified plankton in the guts of juvenile hilsa

There were 26 genera of phytoplankton were recorded in the analysis of juvenile hilsa guts, including Bacillariophyceae (or diatoms; 15 genera), Chlorophyceae (or green algae; 4 genera), Cyanophyceae (3 genera), Dinophyceae (3 genera) and Euglenophyceae (1 genus). 16 genera of zooplankton, including Copepoda (7 genera), Rotifera (4 genera), Cladocera (4 genera) and Tentaculata (1 genus) were also found in the guts of juvenile hilsa (Table 1) in the present study. The sand particles were also observed in the guts of juvenile hilsa. This study found that phytoplankton had a higher generic abundance while zooplankton had a lower generic abundance in the juvenile hilsa gut which has similarities to the study conducted by Hasan *et al*. (2016) [7] and Rahman *et al*. (1992) [14] but they discovered a greater number of plankton genera from the current investigation. They performed a year-long investigation, but the current study only covered five months (July to November), which might explain the reduced number of plankton genera discovered in the current study. In the present study, phytoplankton genera (*Synedra* sp., *Coscinodiscus* sp., *Rhizosolenia* sp., *Microcystis* sp.) and zooplankton genera (*Calanus* sp., *Pseudodiaptomus* sp., *Brachionus* sp.) showed similarity with the findings of Hasan *et al*., (2016) [7].

Table 1: Plankton genera observed under a stereomicroscope in the juvenile hilsa gut analysis

Plankton groups	Genus
Phytoplankton	
Bacillariophyceae	*Synedra, Coscinodiscus, Rhizosolenia, Melosira, Diatoma, Guinardia, Cyclotella, Bacillaria, Thalassionema, Chaeteceros, Nitzschia, Cocconeis, Leptocylindrus, Epithemia, Dinophysis*
Chlorophyceae	*Spirogyra, Ulothrix, Microspora, Muogeotia*
Cyanophyceae	*Microcystis, Trichodesmium, Oscillatoria*
Dinophyceae	*Procentrum, Pyrophacus, Ceratium*
Euglenophyceae	*Phacus*
Zooplankton	
Copepoda	*Calanus, Acrocalanus, Diaptomus, Pseudodiaptomus, Naupleus, Leptodora, Zoea*
Rotifera	*Brachionus, Filinia, Trichocera, Poliarthra*
Cladocera	*Penilia, Bosmina, Daphnia, Diaphanosoma*
Tentaculata	*Cestum*

3.2 Frequency of occurrence (FOC %)

A gradual increasing FOC% rate (July to December) was found in bacillariophyceae, cyanophyceae and chlorophyceae but highest value (100%) was recorded in bacillariophyceae at the months November and December. Copepoda and rotifer were also showed FOC% in almost every month but highest values were recorded in the later months of the study period (Table 2).

Coscinodiscus sp., *Rhizosolenia* sp., *Synedra* sp., *Muogeotia* sp., *Cyclotella* sp., *Microcystis* sp., *Calanus* sp. and *Pseudodiaptomus* sp. were found as the most dominant genus with higher occurrence (Table 2). The mentionable amount of sand particles (5-15%) in the gut content of juvenile hilsa was recognized from July to December (Table 2).

In the size groups of juvenile hilsa, below 5g and 5-10g the highest occurrence was observed for copepod (85.71% and 83.33%) followed by bacillariophyceae (80.95% and 81.77%), cyanophyceae (43.75%, 44.44%), rotifera (34.78% and 38.89%), chlorophyceae (33.33%, 38.89%) and cladocera (4.28% and 2.22%) respectively. The same pattern was also followed by the size group of 10-15g hilsa. But other two size groups (15-20 g and 20-25 g) showed different values (Table 2). Copepod as zooplankton group was showed its higher frequency in the early stages (below 5g, 5-10g and 10-15g) of juvenile hilsa but move to both zooplankton and phytoplankton with their growing ages (15-20g and 20-25g) (Table 2). Bacillariophyceae (diatoms) was the major items in the gut with *Coscinodiscus* sp. (37.61-68.75%), *Rhizosolenia* sp. (23.81-44.44%) and *Synedra* sp. (55.09-87.5%). On the other hand, highest copepod genera were recorded *Pseudodiaptomus* sp. (25-61.11%) and *Calanus* sp. (30.43-75.45%) were found as zooplankton (Table 2).

Table 2: Monthly and size-group wise variation of frequency of occurrence (FOC %) of different food items examined in the gut content of juvenile hilsa.

Genus	Jul	Aug	Sep	Oct	Nov	Dec	<5g	5-10 g	10-15 g	15-20g	20-25 g
Phytoplankton											
Bacillariophyceae	85	85	95	95	100	100	80.95	81.77	88.18	100	100
Synedra	60	55	85	75	70	75	58.90	67.78	55.09	82.6	87.5
Rhizosolenia	45	40	20	25	30	25	23.81	44.44	31.82	43.47	31.25
Cyclotella	25	35	25	70	45	35	28.10	22.22	38.91	47.82	50
Dinophysis	5	10	-	-	15	25	9.52	-	-	17.39	-
Diatoma	10	10	-	15	10	20	-	5.55	9.1	13.04	-
Cocconeis	25	30	25	45	35	15	33.33	27.78	31.36	34.78	37.5
Leptocylindrus	15	30	35	30	35	30	19.05	27.78	-	52.17	56.25
Epithemia	15	-	20	15	30	25	9.52	-	13.64	30.43	25
Coscinodiscus	45	55	40	65	70	65	37.61	50	50	65.21	68.75
Bacillaria	10	15	15	5	20	25	4.76	22.22	15.18	-	25
Chaeteceros	-	10	-	5	10	20	9.52	-	9.1	-	6.25
Nitzschia	-	10	10	10	10	15	9.52	5.55	9.1	4.34	12.5
Melosira	20	-	10	15	20	15	-	-	18.18	26.09	25
Guinardia	10	15	10	10	5	10	7.52	-	13.64	21.74	6.25
Thalassionema	15	15	10	25	15	10	4.76	11.11	22.73	30.43	18.75
Chlorophyceae	35	30	45	40	45	45	33.33	38.89	40.91	41.78	50
Spirogyra	15	10	15	-	5	15	-	11.11	13.64	27.39	25
Muogeotia	25	30	25	35	30	20	28.57	27.78	45.45	39.13	31.25
Ulothrix	10	-	5	10	-	10	14.28	16.67	-	-	18.75
Microspora	-	-	10	15	10	-	-	5.55	9.1	37.39	6.25
Cyanophyceae	40	45	50	60	60	55	43.75	44.44	54.55	56.52	62.5
Microcystis	30	35	35	40	50	35	38.10	38.89	36.36	47.82	37.5
Trichodesmium	25	15	25	15	30	20	28.57	16.67	22.73	26.09	25
Oscillatoria	20	20	25	20	15	20	14.28	22.22	40.91	30.43	37.5
Dinophyceae	25	-	30	45	30	25	0	0	1.36	5.43	7.5
Procentum	5	-	15	10	-	20	0	0	4.55	8.69	12.5
Pyrophacus	20	-	15	30	35	5	0	0	1.82	2.74	-
Ceratium	5	-	-	10	-	5	0	0	-	-	1.5
Euglenophyceae	-	5	20	15	-	-	14.28	-	-	3.04	8.75
Phacus	-	5	20	15	-	-	1.28	-	-	3.04	8.75
Zooplankton											
Copepoda	60	65	70	70	75	70	85.71	83.33	90.91	65.22	50
Calanus	35	55	40	50	55	50	65.91	67.56	75.45	30.43	43.75
Diaptomus	10	15	15	5	20	10	16.28	36.67	52.73	-	-
Naupleus	5	-	-	-	25	5	8.76	5.21	49.21	4.35	-
Pseudodiaptomus	30	50	45	50	50	45	52.38	68.11	59	43.47	25
Acrocalanus	25	20	20	25	15	10	9.52	31.11	49.1	4.35	-
Leptodora	5	5	-	10	15	-	6.76	12.11	52.11	4.35	-
Zoea	10	15	10	5	10	15	4.76	11.11	44.31	8.69	6.25
Rotifera	20	20	20	25	35	30	34.78	38.89	34.78	22.73	12.5
Filinia	-	10	-	5	5	10	7.76	5.55	8.69	-	-
Brachionus	15	10	15	15	25	10	14.52	16.67	13.04	18.18	12.5
Trichocera	-	-	5	-	-	25	-	-	-	4.55	-
Poliarthra	5	-	5	5	5	-	-	16.67	-	-	-
Cladocera	10	20	25	35	25	10	4.28	2.22	3.45	3.04	1.75
Penilia	5	-	-	5	10		3.76	-	3.1	-	1.25
Bosmina	-	10	25	20	15	-	1.52	3.11	2.73	3.04	1.5
Daphnia	5	5	-	10	-	5	-	4.55	1.64	-	-
Diaphanosoma	-	5	-	-	-	5	-	-	-	-	-
Tentaculata	15	-	5	10	5	5	-	16.67	-	8.69	6.25
Cestum	15	-	5	10	5	5	-	16.67	-	8.69	6.25
Unidentified	15	10	10	5	15	5	2.52	1.11	1.73	2.11	1.52
Sand Particles	5	10	5	15	5	5	2.54	3.01	1.74	2.11	1.27

Copepod concentration was found higher than bacillariophyceae in early size groups of hilsa which was supported by Bhaumik *et al*. (2013) [2] and Hasan *et al*., (2016) [7]. De *et al*. (2013) [5] in West Bengal showed different result and mentioned that copepods were the major items in the guts of hilsa from 10 g to 30 g, followed by diatoms which also supported the present study result. The percentage frequency of the existence of sand particles (5-15%) was discovered in the present study result which was also reported by Karna *et al*. (2014) [11].

3.3 Volumetric analysis index (%)

Volumetric index analysis (%) of gut samples indicated the highest volume of bacillariophyceae (66.67% and 61.81%), Cyanophyceae (8.33% and 9.45%) in November and December respectively (Figure 2).

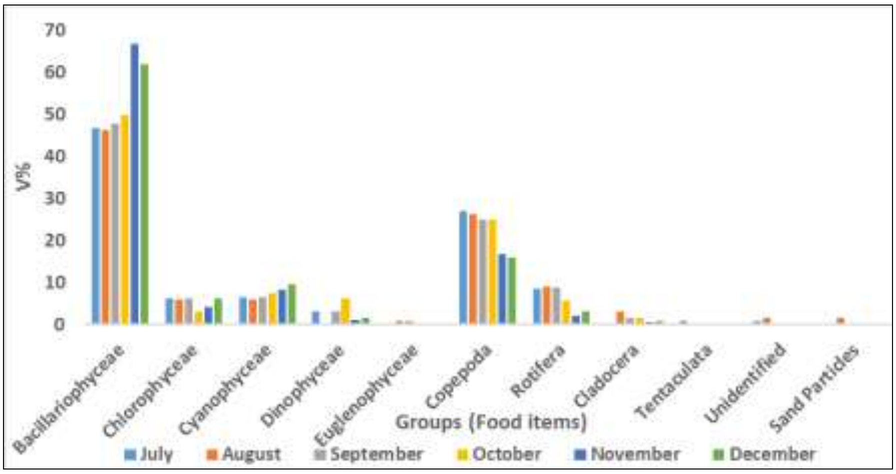

Fig 2: Monthly variation in the composition of food items in terms of volumetric analysis index (%).

On the other hand, zooplankton groups copepod showed higher volume (%V) in July (26.85%) and August (26.06%) and a gradual decrease towards December (15.90%) and the same pattern was also followed by rotifer in the study period (Figure 2). Euglenophyceae (0.38-0.76%) showed a very lower volume in the gut of hilsa. Sand particles observed in all the months of the present study in the gut of juvenile hilsa (0.13% to 1.50%) but did not show any specific pattern among the months (Figure 2). The composition in terms of the volumetric index (%) of different groups of food items of five size groups i.e., below 5g, 5-10g, 10-15g, 15-20g and 20-25g was given in Figure 3. Volumetric index analysis (%) of gut samples (Figure 3) indicated that copepod constituted the higher volume in early age groups (< 5 g), (5-10g) and (10-15g) than other age groups (15-20g) and (20-25g). Bacillariophyceae also occupied the highest volume in the gut sample of juvenile hilsa of all five size groups but the opposite pattern than copepod. In below 5g hilsa gut it occupied (49.42%), 5-10g (46.55%), 10-15g (49.90%), 15-20 (46.72%) and 20-25 (51.2%).

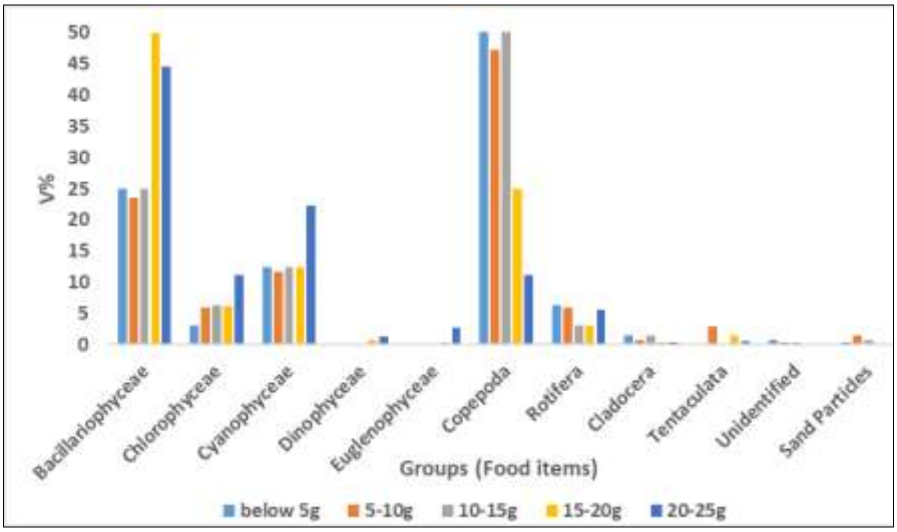

Fig 3: The volumetric analysis index (%) of groups of food items observed in gut content of hilsa of different size groups.

In % V we observed that presence of copepod was observed lower in 15-25g of hilsa. So, in the present study collected hilsa sample (November- December) were mainly this size groups (15-25g) which might be the reason of lower presence of copepode at those months. All the size groups of juvenile hilsa consume higher volume of copepod and bacillariophycea but copepod concentration was recorded higher than bacillariophycea in early size groups (below5g to 15g). That means at very early stages juvenile hilsa consume mainly copepods and with increasing ages they divert their feeding behaviour towards diatoms along with little amount of copepod which was also concluded by De *et al*. (2013) [5].

Sand particles (0.20 to 1.5% of total gut volume) were found in the stomachs of the majority of hilsa in the early stages, as were De *et al*., (2013) [5] (4.17 to 37.50% of total gut volume), and widely differed with the result of present study. This variation might be due to the geographical distribution and study period. Bottom feeding of hilsa in its early phases also observed by Sarker *et al*. (2023) [17]. They revealed that young hilsa aggregate near the bottom and incidentally ingest of sand grains with attached microflora which might be the reason of the presence of sand grains in the gut of juvenile hilsa.

3.4 Relative length of gut (RLG)

To characterize the different sizes of fish as carnivores, herbivores and omnivores were undertaken by using the RLG (RLG) but for juvenile hilsa, it was calculated to know changes in feeding habits of hilsa in different sizes. The relative length of gut (RLG) of juvenile hilsa in different size groups (0.21, 0.23, 0.24, 0.27 and 0.28 of below 5g, 5-10g, 10-15g, 15-20g and 20-25g respectively) was observed from the total length (mm) and total gut length (mm) revealing that the length of the gut was linearly correlated with sizes of hilsa (Table 3). The eating preferences of hilsa may shift from phytoplankton to zooplankton as their relative gut length grows (De *et al*., 2013) [5], supporting the current findings. An increase in the relative length of gut (RLG) of juvenile hilsa from fry to adult is indicative of changes in eating habits in the same environment (De and Dutta, 2011) [4], which might explain the progressive rise in the relative length of gut (RLG) of juvenile hilsa in the current research result.

Table 3: The relative length of gut (RLG) values of all fishes in different size groups

Size Groups	Total Number of fish examined	Total Length (mm)	Total Gut Length (mm)	RLG Value
Below 5 g	21	1485	312	0.21
5-10 g	18	1613	371	0.23
10-15 g	22	2241	546	0.24
15-20 g	23	2517	689	0.27
20-25 g	16	1879	518	0.28

3.5 Index of Relative Importance (IRI%)

Index of relative importance (IRI%) calculations which attempt to convene the numerical, volumetric, and frequency of occurrence measurements into one value, help to rank the food items in the gut of juvenile hilsa. Copepoda with an IRI% of 49.11 to 58.65% constituted the bulk of food items in the gut contents of hilsa, and bacillariophyceae ranked second (25.19 to 32.21%) in all studied months (Table 4). Interestingly sand particles although found in the gut of juvenile hilsa in both methods (frequency of occurrence and volumetric index analysis) yet did not constitute food items due to value absent in IRI% (Table 4). Therefore, the ranking of feeding habits of hilsa based on IRI% was Bacillariophyceae > Cyanophyceae > Chlorophyceae > Dinophyceae > Euglenophyceae; that of zooplankton was Copepoda > Rotifera > Cladocera > Tentaculata (Table 4). % IRI cannot account for sand grains as the plankton samples were not evaluated for sand grain contents and really could not be, as it was impossible to sample sand grains in the water column the way the fish themselves might (Hart *et al*., 2002) [8].

Table 4: Monthly variation of index of relative importance (%) observed in groups of food items in the gut content of juvenile hilsa

IRI (%)	July	August	September	October	November	December	Rank
Bacillariophyceae	25.19	26.21	27	29.10	32.21	30.49	2
Chlorophyceae	3.47	3.00	4.03	2.52	3.66	3.22	5
Cyanophyceae	6.37	6.59	7.97	8.51	8.76	10.37	3
Dinophyceae	1.28	-	1.54	3.54	2.08	1.09	6
Euglenophyceae	-	0.03	0.50	0.25	-	-	8
Copepoda	58.65	58.51	56.81	53.95	50.52	49.11	1
Rotifera	2.84	1.04	0.93	0.59	0.65	0.45	4
Cladocera	0.38	0.80	0.68	1.58	0.70	0.90	7
Tentaculata	0.07	-	0.05	0.08	0.06	0.04	9

4. Conclusion

This study examined the monthly and size variation of feeding preferences of juvenile hilsa fishes from Meghna river estuary in Moju Chowdhury hat, Lakshmipur**,** Bangladesh. All of the fish studied were classified into five size groups: size group 1 (below 5g), size group 2 (5-10g), size group 3 (10-15g), size group 4 (15-20g), and size group 5. (20-25g). A total of 26 microalgae and 16 zooplankton genera were recorded from the gut contents of juvenile hilsa. Bacillariophyceae was most important in terms of percent frequency (%F) and percent volume (%V) in the stomachs across all hilsa size groups, whereas zooplankton occurred more frequently in the smaller sizes of hilsa. Copepods ranked first, and diatoms ranked second in importance as dietary items based on the index of relative importance (IRI%). A progressive rise in relative length of gut (RLG) of juvenile hilsa is indicative of changes in eating habits in the same environment. In the present study result, according to FOC% and V%, we found sand particles as a constituent in the gut of juvenile hilsa but from % IRI value it was observed that sand was only engulfed by juvenile hilsa with food particles during bottom feeding but not a preferred food item for juvenile hilsa.

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