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Monthly analysis of gut content of near- threatened *Nandus nandus* (Hamilton, 1822) at Kawadighi *Haor* in northern Bangladesh

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ABSTRACT

The successful fish species culture is correlated with the profound analysis of the food and feeding habits of that species. This research aimed to address the monthly variation in the gut contents of *Nandus nandus*. A total number of 108 specimens were collected from Kawadighi Haor in Sylhet division, Bangladesh from January to December 2014. Four taxa of zooplankton were identified, belonging to Crustacea (9 genera), Rotifera (6 genera), larvae (2 genera), and tiny fish and small prawns (3 genera), in addition to three classes of phytoplankton *viz*. Bacillariophyceae (2 genera), Chlorophyceae (6 genera), and Cyanophyceae (2 genera) were recorded from the guts. Crustacea ($6.00\pm0.62\times10^3$ units/l) showed the dominant group among the zooplanktons, while Chlorophyceae ($3.14\pm0.49\times10^3$ units/l) was the most abundant among phytoplanktons. The average index of gut fullness recorded its highest in March (3.67) and the lowest in December (2.11). All fish were actively fed during the whole year, and the percentage of empty guts was almost non-existent. Based on the present findings, zooplankton was the most preferred feed for *N. nandus* although it fed on both phytoplankton and zooplankton. The current data provide comprehensive data that would support the management and conservation of the species under study through the domestication of the near-threatened *N. nandus* in the open waters of Bangladesh, paving the way for aquaculture in captivity.

INTRODUCTION

Among 260 freshwater fishes of Bangladesh, over 150 are small indigenous species (SIS) **(Amin *et al*., 2009)**. *Nandus nandus* **(Hamilton, 1822)**, also known as bheda, vera, meni, roina in Bangladesh, is one of the important SIS with an attractive taste and high market price for its compact muscle and less bony structure. The crude protein, fat, carbohydrate, and mineral contents in *N. nandus* are 52.50%, 2.00%, 0.21%, and 5.70%, respectively **(Ray & Dhar, 2012)**. This species is widely found in India, Pakistan, Bangladesh, Nepal, Myanmar and Thailand **(Talwar & Jhingran, 1991; Rahman,**

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2005). It is a carnivorous fish feeding on tiny shrimp and fish, insect larvae and other small animals **(Mustafa *et al*., 1980; Saha *et al*., 2002)**. It's camouflage capacity to change its body color against its environment helps better predation **(Dutta *et al*., 2013)**. The fish mainly feeds on the bottom and in the columns **(Mustafa *et al*., 1980)**. The fish species grows up to 20cm in length and is found in ditches, flooded fields, *beel* and small creeks **(Talwar & Jhingran, 1991)**. This fish usually breeds from April to September **(Hossain & Afroz, 1991)**. In Bangladesh, several populations of indigenous fish species have witnessed a decline, among which *N. nandus* fish are considered. **IUCN Bangladesh (2015)** enlisted this species under the red list of near- threatened fishes in Bangladesh, and thus was globally considered of the least concerned **(Ng, 2010)**. This rank was obtained though it was considered vulnerable just one decade before **(IUCN Bangladesh, 2000)**. Consequently, further studies are highly recommended to conserve this species, especially artificial breeding. However, to accomplish this purpose, *N. nandus* fish requires domesticity, in which knowledge about feeding biology is demanded. several researches have been conducted on different aspects of this species, such as histology, morphological and allozyme variation, biology, etc. **(Goswami and Dasgupta, 2004; Zohora *et al*., 2010; Rahman *et al*., 2012)**. Nevertheless, limited works on its gut contents have been initiated **(Parameswaran *et al*., 1971; Paul, 2020)**. Therefore, gut content analysis is essentially needed for the conservation and domestication of this species. In this respect, the current study was organized to address the food habits of this near-threatened fish, hoping that the findings would assist in the effective management of wild *N. nandus* populations and pave the way for domestication.

MATERIALS AND METHODS

1. Study Site

The research work was conducted at Kawadighi Haor, located at Rajnagar Upazila (sub district) under Moulvibazar district of Sylhet division, Bangladesh. The waterbody lies between 24°26´ and 24°39´ north latitudes and between 91°44´ and 91°58´ east longitudes (Fig. 1).

2. Sampling of Fish

An approximate number of nine fish specimens were randomly collected once a month from local fishers of Kawadighi Haor from January to December 2014. Fish samples were preserved in 10% formalin in a plastic bottle immediately to prevent digestion of food components and halt the enzymatic activity of the digestive tract. The fish samples were brought to the laboratory at the Department of Aquaculture, Sylhet Agricultural University, Sylhet, Bangladesh at the earliest convenience (Not more than 3 hours). The collected fishes ranged from 10.96 cm to 13.87 cm in length and varied from 26.86 gm to 51.23 gm in body weight.

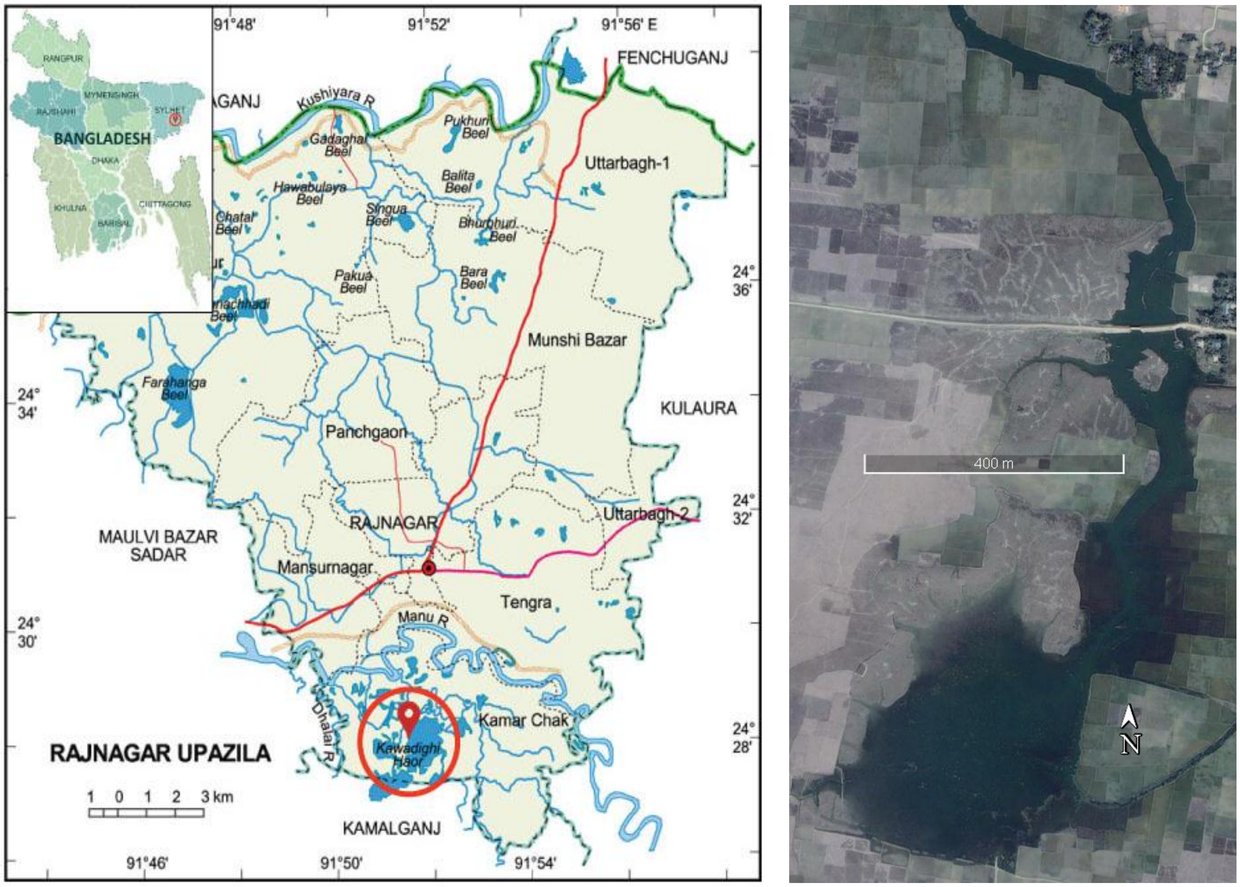

Fig. 1. The location of Kawadighi Haor (Google Earth Pro)

3. Microscopic Observation of Gut

The whole guts were separated from the fish body and dissected with sharp scissors. Gut contents were put in a clean petridish containing 10% formaldehyde. Food contents were taken out and diluted in 5 ml distilled water using the methods of **Dewan *et al.* (1985)** and **Miah and Siddique (1992)**. A pipette was used to transfer an amount of 1 ml sub-sample to a Sedgwick-Rafter cell. For microscopic examination, ten fields were chosen randomly from 1,000 fields in the counting cell. Then, the total number of planktons detected in the selected 10 fields were counted.

By using a binocular microscope (Olympus, model-CX41RF, Japan) all organisms were identified to a genus level using identification keys of **Needham and Needham (1941)**, **Prescott (1962)**, **Belcher and Swale (1976)** and **Bellinger (1992)**.

The calculation of plankton of concentrated sample is performed using the following formula:

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where,

N = Number of plankton cells or unit

A = Total number of plankton counted
C = Volume of final concentrated sample in ml
V = Volume of a field in mm^3
F = Number of fields counted
L = Volume of original water in liter

Index of fullness method was also used to observe the feeding intensity. In fullness method, an index of fullness of the gut was recorded according to the size of the stomach of the individual fish, giving score 0 for empty, 1 for ¼ full, 2 for ½ full, 3 for ¾ full and 4 for full stomach as suggested in the study of **Pillay (1952)**.

4. Data Analysis

Microsoft Excel version 2010 was used to analyze the data. Results were presented as percentage, $\times10^3$ units/litre, and mean ± standard deviation.

RESULTS

1. Gut Contents of *N. nandus*

The guts of the examined samples contained eight major groups of organisms from phytoplankton, zooplankton, small prawns and small fishes. Three groups were phytoplankton, *viz*. Bacillariophyceae, Chlorophyceae, and Cyanophyceae. Among six groups of plankton, three groups were zooplankton; namely, Rotifera, larvae and Crustacea.

1.1 Zooplankton Detected in the Gut of *N. nandus*

Three zooplankton groups were recorded from the gut content; namely, crustacean, rotifer and insects larvae. However, in the current study, small crustaceans and their fragments formed the significant proportion of the gut content and contribute to a percentage of 65 of the zooplankton, while rotifers contributed 31% (Fig. 2). Insect larvae formed another group of zooplankton, covering only 4% of the food content among zooplankton (Fig. 2).

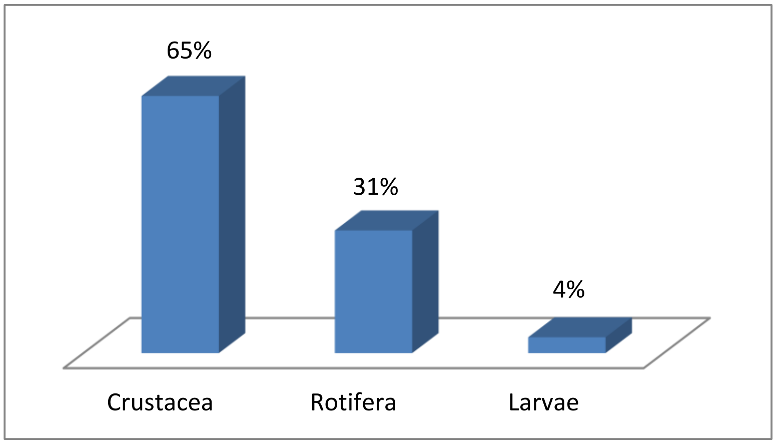

Fig. 2. Percentages of different zooplankton groups found in the gut of *N. nandus*

1.1.1 Crustaceans

In total, 9 genera under Crustacea were identified, *viz*. *Cyclops*, *Cypris*, Nauplius larva, *Bosmina*, *Daphnia*, *Diaphanosoma*, *Sida*, *Moina*, and *Diaptomus* in the diets of fish, which were present regularly around the year. In this study, the maximum number of crustaceans was observed in March ($6.65 \pm 0.62 \times 10^3$ units/l) and September ($6.6 \pm 0.62 \times 10^3$ units/l) and suddenly decreased in the subsequent months, including October, November, and December. Whereas, the lowest amount of crustaceans was identified in December ($4.55 \pm 0.62 \times 10^3$ units/l) (Fig. 3).

1.1.2 Rotifera

Six genera; namely, *Brachionus*, *Trichocerca*, *Asplanchna*, *Notholca*, *Keratella*, and Filinia were identified in the diets of fish during the whole year. The occurrence of rotifers reached its maximum (3.4±0.39×103 units/l) in March and minimum (2.15±0.39×103 units/l) in December (Fig. 3). These six genera remained similar quantity throughout the study period except some little decreased in June, August, and December.

1.1.3 Larvae

Maximum occurrence of insect larvae (Chironomid and notonectidae) was recorded in February and March (0.5±0.07×103 units/l) and minimum observed in June, November, and December (0.3±0.07×103 units/l) (Fig. 3).

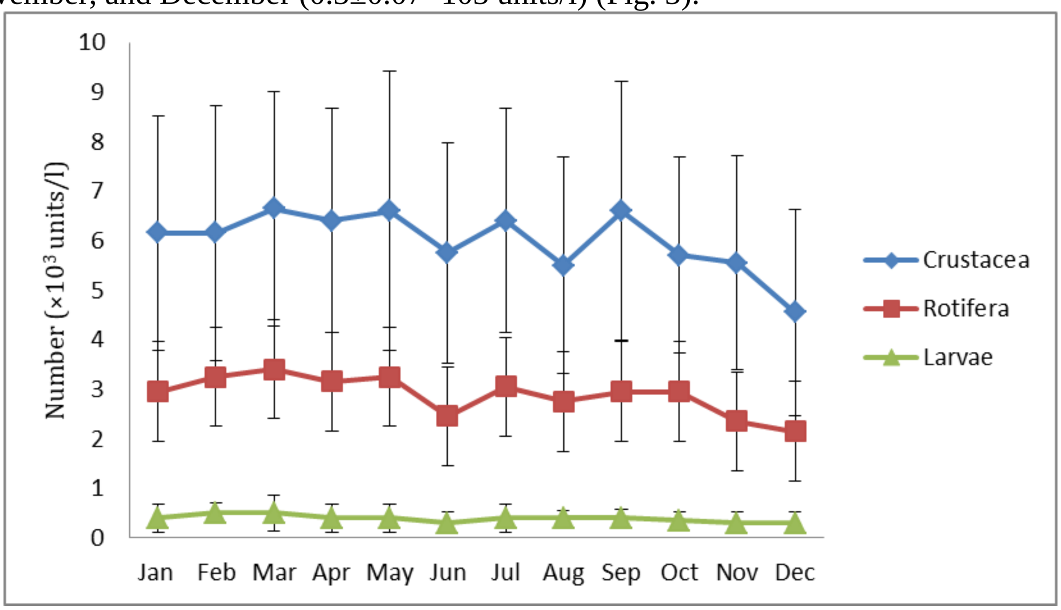

Figure 3. Monthly change of Crustacea, Rotifera, and Larvae

1.2 Fishes and small prawns found in the gut of *N. nandus*

Two species of fishes, namely *Chanda nama*, *Puntius ticto*, and small prawn (*Macrobrachium* spp.), were found in the gut of *N. nandus* over the year. In this study, the maximum predation of tiny fish content in *N. nandus* was observed in March, April, September, and November, and no fish/body parts content was observed in May (Fig. 4). Maximum predation of small prawns was observed in February, June, and September, and no fish or prawn content was observed in May, August, and December (Fig. 4).

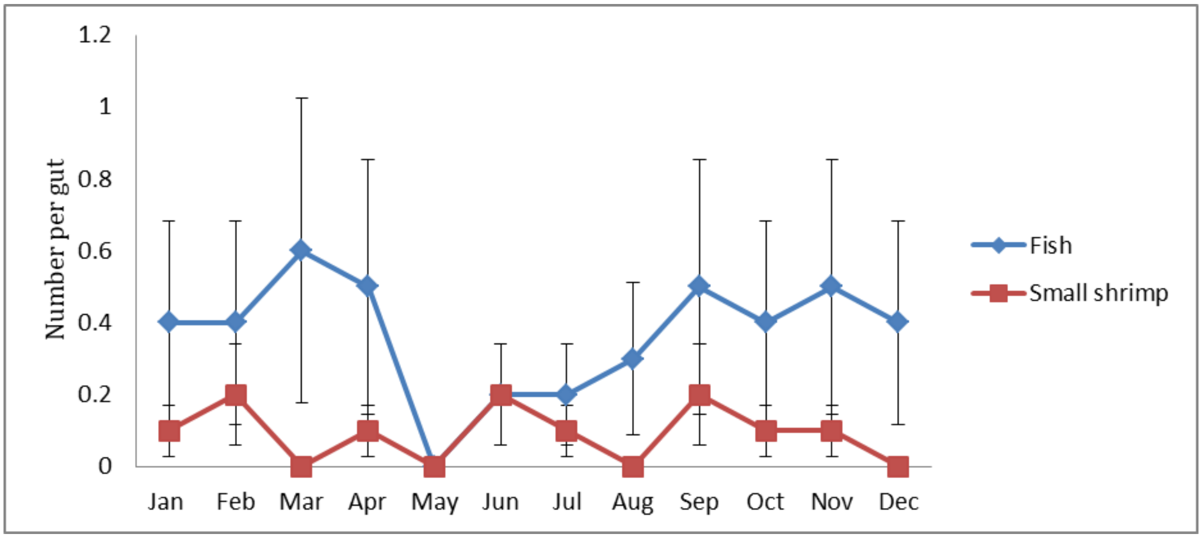

Figure 4. Fish and small prawn consumed by *N. nandus* over the study period

1.3 Phytoplankton Found in the Gut of *N. nandus*

A total number of 9 genera of phytoplankton belonging to 3 classes (Chlorophyceae, Bacillariophyceae, and Cyanophyceae) were identified from the gut contents of the examined fishes. Chlorophyceae was the dominant phytoplankton group (Fig. 5).

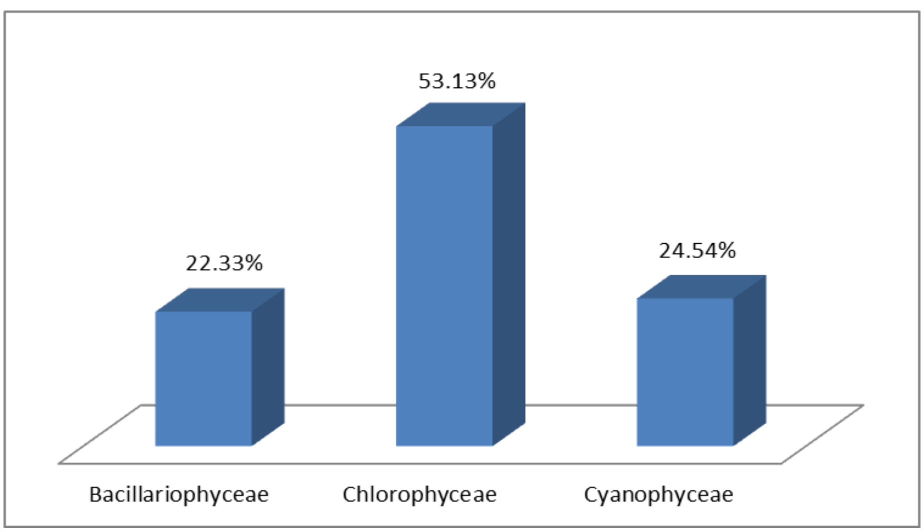

Figure 5. Comparison of different phytoplankton groups (%) found in the gut of *N. nandus*.

1.3.1 Chlorophyceae

The phytoplankton belonging to Chlorophyceae was the dominant group in diets. There were five genera such as Ankistrodesmus, Chlorella, Closteridium, Scendesmus, and Oscillatoria, and formed the most abundant group and made up (53.13%) of the gut contents among the phytoplankton (Fig. 5). Maximum occurrence of Chlorophyceae ($4\pm0.49\times10^3$ units/l) was observed in September and minimum ($2.6\pm0.49\times10^3$ units/l) in August (Fig. 6).

1.3.2 Cyanophyceae

In this study, two genera represented this group and were found to occur throughout the study period. Among the phytoplankton, Cyanophyceae was found in the second position and made up 24.54% of gut contents (Fig. 5). Two genera such as Anabaena and Microcystis, were identified under this group. The genera under Cyanophyceae was relatively more encountered in March and July (Fig. 6). The minimum occurrence was observed in the month of December ($0.95\pm1.45\times10^3$ units/l).

1.3.3 Bacillariophyceae

Among the phytoplankton groups, Bacillariophyceae was found to stand the third position in terms of number in the gut. There were two genera *viz*. Cyclotella and Cosmarium. In the current study, Bacillariophyceae constituted 22.33% of the gut contents among the phytoplankton (Fig. 5). In Fig. 6 occurrence of Bacillariophyceae gradually increased from January and reached maximum occurrence ($1.6\pm0.24\times10^3$ units/l) in the month of July with some exception in May. Notable decrease in the subsequent months and minimum was found in April, May, and December with an average value ($1.32\pm0.24\times10^3$ units/l).

2. Degree of Feeding

Gut content analysis revealed that their feeding habits changed over time. The dietary differences might indicate that these individuals lacked selectivity, opting for a more generalist approach to food choices. Percentage of empty gut were almost absent despite a little except in August and December. Proportion of full gut was noticed higher in March (66.67%) and lowest in August, November and December (11.11%) (Table 1). The average index of fullness was height at March (3.67) and lowest at December (2.11) (Table 1). According to feeding intensity percentage it can be concluded that almost all fish were fed actively all the year round.

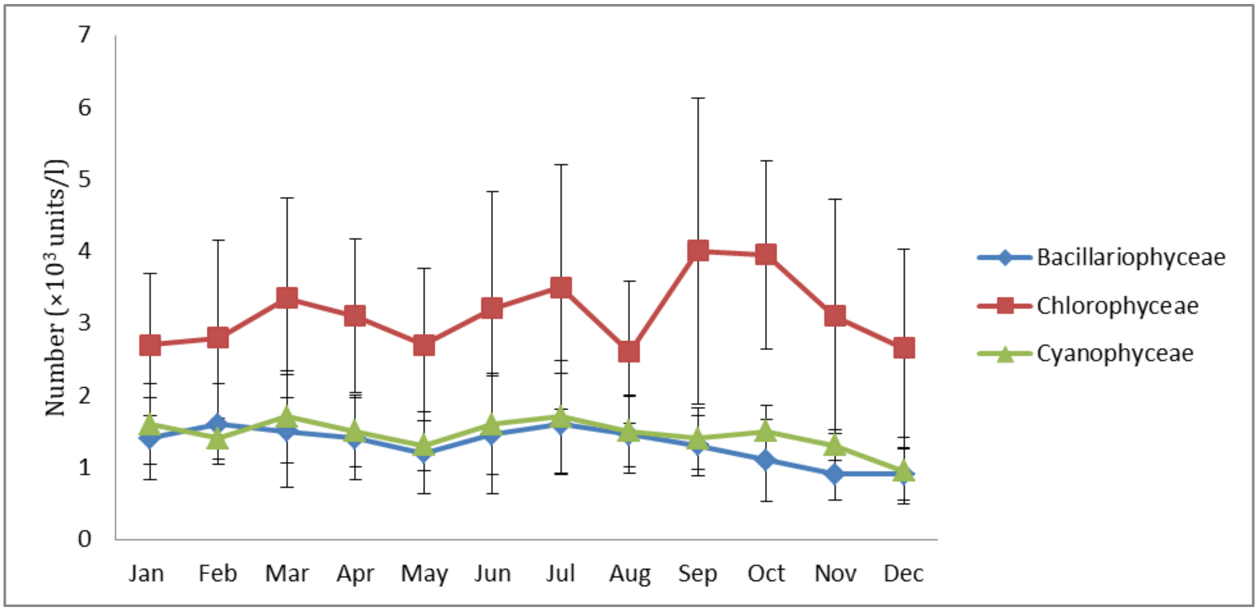

Figure 6. Monthly change of Bacillariophyceae, Chlorophyceae and Cyanophyceae

Table 1. Feeding intensity percentage and Average index of fullness

Month	Number of fish examined	Feeding intensity %					Average index of fullness
		Full	3/4 full	1/2 full	1/4 full	Empty	
Jan	9	33.33	33.33	22.22	11.11	0.00	2.89
Feb	9	55.56	11.11	22.22	11.11	0.00	3.11
Mar	9	66.67	33.33	0.00	0.00	0.00	3.67
Apr	9	44.44	33.33	22.22	0.00	0.00	3.22
May	9	44.44	22.22	33.33	0.00	0.00	3.11
Jun	9	33.33	44.44	22.22	0.00	0.00	3.11
Jul	9	44.44	44.44	11.11	0.00	0.00	3.33
Aug	9	11.11	44.44	33.33	0.00	11.11	2.33
Sep	9	55.56	44.44	0.00	0.00	0.00	3.56
Oct	9	33.33	44.44	11.11	11.11	0.00	3.00
Nov	9	11.11	22.22	44.44	22.22	0.00	2.22
Dec	9	11.11	22.22	44.44	11.11	11.11	2.11

DISCUSSION

The current study examines the gut content of the near-threatened small fish *N. nandus*. The guts of the examined samples contained three groups of zooplankton, namely Rotifera, Larvae, and Crustacea, three groups of phytoplankton *viz*. Bacillariophyceae, Chlorophyceae, and Cyanophyceae, two species of fish and small shrimp. *N. nandus* as a carnivorous fish that feeds on small shrimps, fishes, insect larvae, and other small creatures **(Mustafa *et al*., 1980; Saha *et al*., 2002)**. However, due to a lack of in-depth research, little published data on the gut content of the same species are available. Dominant food items were fish fingerlings, insects, crustaceans, gastropods, eggs, and aquatic vegetation in the case of carnivorous fish *Channa punctatus* **(Bais *et al*., 1994)**. **Azadi *et al*. (1987)** reported two groups of phytoplankton *viz*. Chlorophyceae, Bascillariophyceae in the guts of *Mystus vittatus*. In *Mystus vittatus* **Victor Raj *et al*. (2014)** discovered three zooplankton groups, while **Azadi *et al*. (1987)** recognized six genera. In the case of *Gudusia chapra* in an earthen pond, **Alam *et al*. (2002)** found seven species of zooplankton. **Roy *et al*. (2013)** identified four groups of zooplankton, namely protozoans, rotifer, crustaceans, and insects, in the case of climbing perch collected from north eastern *Haor* basin.

However, in this study crustaceans contribute 65%, Rotifera 31%, and Larvae contribute 4% of the food content among the zooplankton (Fig. 2). **Parameswaran *et al*. (1971)** reported that larger post-larvae of *N. nandus* consume crustaceans (Cladocerans) in large amounts (53.09%). **Bhuiyan and Rahman (1983)** found *C. gachua* a carnivorous fish predominately consumed crustaceans (21.03% of total food contents). Nine genera under Crustacea were identified, *viz*. Cyclops, Cypris, nauplius, Bosmina, Daphnia, Diaphanosoma, Sida, Moina, Diaptomus in the diets of fish. **Parameswaran *et al*. (1971)** identified four genera of Cladocerans (Moina, Bosmina, Simocephalus and Ceriodaphnia) in the gut content of *N. nandus*. **Roy *et al*. (2013)** identified four genera *viz*. Daphnia, Cyclops, Cypris and Microthrix in the gut of *Anabas testudineus* and *Rita rita*. **Chowdhury *et al*. (2007)** found 11 genera of zooplankton, among which 6 belonged to Crustacea and 5 belonged to Rotifera in case of open waters. In this study, the maximum number of crustaceans was observed in March ($6.65\pm0.62\times10^3$ units/l) and September ($6.6\pm0.62\times10^3$ units/l) and the lowest amount of crustaceans was identified in December ($4.55\pm0.62\times10^3$ units/l) (Fig. 3). **Victor Raj *et al*. (2014)** reported that the percentage composition of crustaceans in the case of *M. vittatus* was recorded highest during March 2010 (28.87%) and the lowest in December 2009 (0.23%). Although crustaceans found in the gut and fluctuating all the year round. High water turbidity, water temperature, seasonal variation might be the cause of zooplankton fluctuation in the gut contents of *N. nandus*.

Six genera of Rotifera, namely Brachionus, Trichocerca, Asplanchna, Notholca, Keratella, and Filinia, were identified in the diets of *N. nandus* round the year. **Parameswaran *et al*. (1971)** identified four genera (*viz*. Brachinous, Keratella, Conochilus, and Polyarthra) of rotifers in the gut of *N. nandus*. Rotifers occurred maximum ($3.4\pm0.39\times10^3$ units/l) in March and minimum ($2.15\pm0.39\times10^3$ units/l) in December (Fig. 3). In the instance of *R. rita*, **Noor *et al*. (2013)** found that the highest percentage of rotifers was found in May and the lowest in January.

Maximum occurrence of fish and prawn was recorded in February and March ($0.5\pm0.07\times10^3$ units/l), while the minimum was observed in June, November, and December ($0.3\pm0.07\times10^3$ units/l) (Fig. 4). This group made up 4.00% of gut content among zooplankton (Fig. 2). **Goswami (2007)** recorded *N. nandus* that consumed insect larva with 2.26%. **Roy *et al*. (2013)** identified insect pupae and ants with 9.90% for *A. testudineus*. **Mustafa *et al*. (1981)** investigated the seasonal variations of feeding habits of *Colisa fasciata* and observed that the fish consumed insect larvae (22.44%) only in summer.

Two species of fishes; namely, *Chanda nama* and *Puntius ticto* in addition to small prawns (*Macrobrachium* spp.) were found in the gut of *N. nandus* over the year. **Goswami (2007)** detected the whole prawn body and/or fish body parts in the gut of *N. nandus*. **Noor *et al*. (2013)** observed tiny fishes, crustaceans, and insects as the major food of predatory fish *R. rita*. Fishes, protozoans, crustaceans, and insects were the most important dietary items for adults *Ompok pabda* in the study of **Bhuiyan and Islam (1991)**. There is no published information on the predation habits of this significant Bangladeshi native fish. An isolated research **(Goswami & Dasgupta, 2004)** studied the biology of *N. nandus,* including its food habit.

Chlorophyceae were the dominant group (53.13% of the gut contents among the phytoplankton) in diets of *N. nandus* (Fig. 5). Five genera, such as Ankistrodesmus, Chlorella, Closteridium, Scendesmus, and Oscillatoria were recorded. **Parameswaran *et al*. (1971)** identified Chlorophyceae as a dominant group and recorded 4 genera (Cosmarium, Staurastrum, Arthrodesmus and Closterium) in the case of *N. nandus*. **Victor Raj *et al*. (2014)** identified 15 genera in the gut content analysis of *M. vittatus*. Maximum occurrence of Chlorophyceae ($4\pm0.49\times10^3$ units/l) was observed in September and the minimum ($2.6\pm0.49\times10^3$ units/l) was in August (Fig. 6). **Roy *et al*. (2013)** identified the highest percentage of occurrence of algae in September for *A. testudineus*. **Chowdhury *et al*. (2007)** found that the occurrence of Chlorophyceae was most abundant in October, while its lowest was detected in April in the case of open water.

Among the phytoplankton, Cyanophyceae was found in the second position (24.54% of the gut content) (Fig. 5). Two genera, Anabaena and Microcystis, were identified under this group. **Parameswaran *et al*. (1971)** identified two genera (Anabaena and Microcystis) of blue-green algae in the gut content of *N. nandus*. The genera under Cyanophyceae was relatively more encountered in March and July. The minimum occurrence was observed in the month of December ($0.95\pm1.45\times10^3$ units/l) (Fig. 6). **Roy *et al*. (2013)** found the maximum percentage of occurrence of algae in September for *A. testudineus;* while in October it was for *R. rita*, but food items were very common in the months of July to September. According to **Chowdhury *et al*. (2007)**, Cyanophyceae abundance was at its highest in September and its lowest in December-January, which partially agrees with the result of the present study.

In the current study, Bacillariophyceae was the third position (22.33% of the gut contents among the phytoplankton) in terms of number in the gut (Fig. 5). There were two genera *viz*. Cyclotella and Cosmarium. **Shafiqul (2000)** investigated the food and feeding habits of dhela (*Osteobrama cotio*) and found that the percentage of

Bacillariophyceae was 17.57% of main food items. In Fig. (6), the occurrence of Bacillariophyceae gradually increased and reached its maximum ($1.6 \pm 0.24 \times 10^3$ units/l) in July. Notable decrease in the subsequent months was recorded in April, May, and December with an average value of $1.32 \pm 0.24 \times 10^3$ units/l. **Rahman (2013)** studied the gut content of spotted snakehead *C. punctata* and recorded the maximum occurrence of Bacillariophyceae in September and the minimum in April. **Chowdhury *et al*. (2007)** postulated that, the Bacillariophyceae showed its maximum in November and its minimum in March in the case of Borobila *Beel* in the Rangpur district of Bangladesh. However, it was noticed that the percentage of Bacillariophyceae in the gut contents of different fishes fluctuated over the different months to a great extent. Low food selection, nutrient accumulation due to higher rainfall, siltation, turbidity, photoperiod, temperature fluctuation, and many other Physico-chemical factors might be the possible causes of fluctuating plankton in the gut. Thus, the stomach of fishes caught in the morning contained more food. This would indicate that these fishes were fed after the lights out.

According to the gut fullness index, except in August and December, the percentage of the empty stomach was essentially non-existent, and all fish were actively fed all year-round. The stomach of fishes caught in the morning contained more food, indicating that these fishes were fed during nighttime.

The findings of the current study are comparable to those of **Reddy and Rao (1987)**, who investigated *M. vittatus*, and the rate of feeding was determined to vary seasonally. Adult fishes were discovered to be underfed during the breeding season, but juvenile fishes were actively fed throughout the year. In this context, **Frost (1945)** assessed that many fish change their diet as they get older.

The food habit of *N. nandus* was also reported by **Mustafa *et al*. (1980)**. The food habit of some other similar carnivoruous small fishes are reported by some researchers like **Bisht and Das (1981); Bhuiyan and Rahman (1983)** for *C. gachua*, **Reddy and Rao (1987)** for *M. vittatus*, **Khan e*t al.* (1988)** for *M. nemurus*; **Bais *et al.* (1994)** for *C. punctatus* and **Ali *et al*. (2003)** for several species of Mastacembelidae. According to the findings, the amount of different food groups in the gut contents of the fish fluctuated monthly, and the fish had a seasonal predilection for particular food groups. The *N. nandus* prefer to eat insects and fish larvae, crustaceans, rotifers, fish, small prawns and poorly preferred different phytoplankton as it is a carnivorous fish.

Based on the facts provided thus far, it can be concluded that post larval stages prefer planktons, particularly zooplankton. Previous study has not established any special preference of these two phases for any single food item, hence specific information on the most desired food items of juveniles and adults is unavailable. Some isolated studies are enlisted in Table 2.

Table 2. Specific preference on some selected food items at juveniles and adult stages of *N. nandus*

Authors and year of the study	Key findings
Parameswaran *et al*. (1971)	Insects as the mostly preferred food items for the juveniles *N. nandus*
Goswami (2007)	Prawns and fishes as the dominant food for the juveniles *N. nandus*
Parameswaran *et al*. (1971)	Adult *N. nandus* showed maximum preference for small fishes
Mustafa *et al*. (1980)	Adults showed maximum preference for prawns and fishes
Rain both (1996)	Documented aquatic insects and fishes as the most preferred food items for adults
Ray (2013)	Enlisted prawns and snails for the adult
Agarwal and Sharma (1966)	Documented insects and crustaceans as the most preferred food items for the adults
Goswami (2007)	Reported maximum preference for some organic matter and prawn (though no such proper information has been documented on this organic matter) in their adult stage

Though there has been recorded stage-by-stage variation in food choice for this fish species, additional research is needed to discover the most favored food items at different phases, particularly the juvenile and adult stages, since this knowledge is critical for captive culture.

CONCLUSION

The *N. nandus* fishery is focused entirely on capture; no culture procedures have been developed for this species yet, and it used to be sold at fish markets alongside other mainly small fish species. To ensure the fishery's long-term viability, it's critical to develop some appropriate culture approaches for this fish species, as well as standardize captive breeding practices. It has already been suggested as a promising freshwater aquaculture candidate. To succeed in the captive culture of any particular fish species, information on foodstuff, feeding behavior, and reproductive biology are essential. The proper application of the information currently available on *N. nandus* food, feeding habits, and reproductive biology, followed by additional studies to fill in the information gaps, will be beneficial in terms of initiating its culture and increasing the success rate in captive breeding in the coming days. Biological management of open water bodies, such as establishment of sanctuaries, protecting overfishing, stopping the release of exotic fish, as well as conservation and domestication of *N. nandus*, are essential for enhancing sustainable production.

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