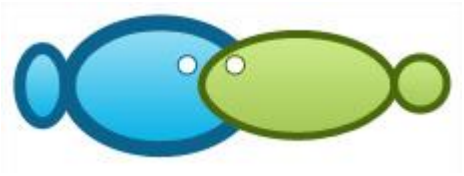

# Biological aspects of three endangered species of *Barilius* from the Atrai River, Dinajpur, Bangladesh

[1]Mosammat Iffat-Ara, [2]Rubaiya Pervin, [3]Nipa Gupta, [1]Mosammat B. Akter, [1]Sharmin Ahmed, [3]Zubaida P. Patwary, [1]Imran Parvez

[1] Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh; [2] Department of Fisheries Management, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh; [3] Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Corresponding author: I. Parvez, iparvez.fbg@hstu.ac.bd

**Abstract**. Along with other genus of Cyprinidae family of fish, *Barilius* has drawned considerable interest owing to high nutritional and commercial value. The present study describes the length-weight relationship, as well as the condition factor and food habit of three *Barilius* spp. from the Atrai river, Dinajpur, Bangladesh. A total of 243 individuals of three species of *Barilius* were randomly collected from fishermen's catch during November 2015 to April 2016. The value of regression coefficient (b) for *Barilius bendelisis*, *Barilius barna* and *Barilius tileo* were 2.77, 2.76 and 2.62, respectively, indicating slightly negative allometric growth pattern. All three species exhibited higher condition factors (K) in February, which were 1.245±0.084, 1.201±0.110 and 1.258±0.249 for *B. bendelisis, B. barna and B. tileo*, respectively. The gut content analysis of *Barilius* spp. suggesting that that these species are omnivore feeders, as plant and animal origin feed were identified. The gut fullness showed almost similar highest proportion of gut and was 34.29% for *B. bendelisis*, 35.48% for *B. barna* and 31.43% for *B. tileo*. The highest mean relative length of gut (RLG) was 2.01±0.21, 1.93±0.123 and 1.78±0.21 for *B. bendelisis, B. barna* and *B. tileo*, respectively, which supported the omnivore feeding hypothesis. The present findings of *Barilius* spp. will be useful in future analyses of other populations and in the stock assessment for their conservation and culture.

**Key Words:** condition factors, food habits, length-weight relationships.

**Introduction**. *Barilius* is a large genus of cyprinid fishes, distributed throughout Bangladesh, India, Nepal, Myanmar, Pakistan, Thailand and Sri Lanka. About 31 species have been identified from different countries. Among them 6 species belonging to the genus *Barilius* have been reported from the stream and rivers with rocky bottom of Dinajpur, Rangpur, Mymensingh and Sylhet regions of Bangladesh (Rahman 2005). These small indigenous species (SIS) are considered as a very important source of essential macro and micro nutrient (Kongsbak et al 2008) and commercially very important due to their palatable taste. The hill trout *Barilius bendelisis* (Hamilton) has drawn the attention as one of the potential candidates for aquaculture and captive breeding in hilly areas of North East India (Suresh & Mandal 2001). However, in recent times, these species are considered as endangered in Bangladesh (IUCN 2015). The possible threats to these species are mainly the over-exploitation and the habitat destruction due to natural phenomena and to certain human activities.

In Dinajpur district, several shallow rivers providing the suitable bed for some unique freshwater small indigenous species (SIS) of fish, including *B. bendelisis*, *Barilius barna* and *Barilius tileo* (Amin et al 2010). Studies about fish biology and ecology improve the fishery management and conservation of threatened fishes. The condition of a fish reflects recent physical and biological circumstances, as it is strongly influenced by

both biotic and abiotic environmental variables, and it fluctuates by interaction among feeding habits, parasitic burden and fish physiological conditions (Le Cren 1951).

Usually, the length-weight relationships (LWRs), condition factors (K), food habit and feeding ecology are studied to determine the fish condition. Application of the LWRs in sustainable fishery management and conservation of natural fish populations are described by several workers from different countries (Pervin & Mortuza 2008; Sarkar et al 2009; Muchlisin et al 2010; Keivany et al 2015; Qamar et al 2017). The isometric and allometric relationships based on regression analysis are often used to estimate fish body forms whereas the condition factor (K) is used to describe the state of well-being of the fish (Bagenal 1978; Zamani-Faradonbe et al 2015). Moreover, the condition factor (K) is helpful in assessing the food supply, timing and duration of breeding of a fish population (Weatherly 1972). In addition, gut content analysis of any species helps to identify the available feed in the river as well as to determine the food habit of species that have a culture potential. Hence, in this study attempts were taken to understand the aquatic ecosystem of the Atrai river's flows in Dinajpur, Bangladesh, by analyzing the length-weight relationship, condition factor, gut content, gut fullness and relative length of the gut (RLG) of three endangered species *B. bendelisis, B. barna* and *B. tileo*.

## Material and Method

***Study sites***. The Atrai River is one of the perennial rivers in northern parts of Bangladesh, flowing from Kumarganj and Balurghat in Dakshin Dinajpur, West Bengal, India. It enters into the Bangladesh and splits into two rivers; the Gabura and the Kankra in Dinajpur district. The river serves as a perennial source of fishing, even though it is often the cause of flooding in many areas during monsoons. The samples were collected from the Atrai river located in Ramdubi, Chirirbandar (25°39'N-88°46') under the district of Dinajpur, Bangladesh (Figure 1).

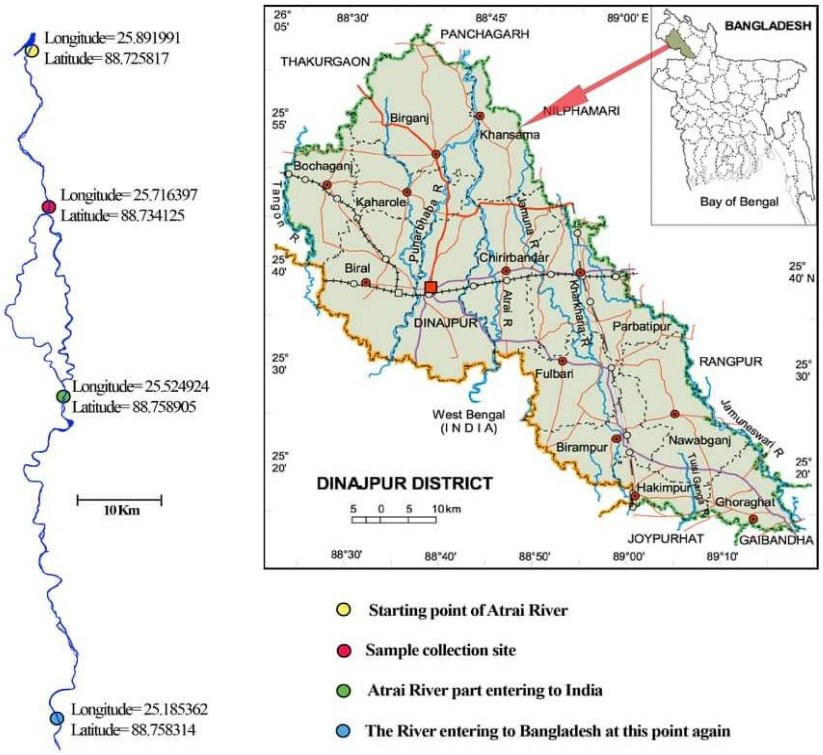


Figure 1. Map of sample collection site in Chirirbandar Dinajpur.

***Sample collection***. The samples of *B. bendelisis, B. barna* and *B. tileo* were collected from fishermen's catch in the Atrai river from November 2015 to April 2016. As all three fish species are considered as endangered, no direct fishing was done for collecting sample. The fish samples were preserved in plastic containers with 10% formalin and

transferred to the laboratory. During the collection, transportation and dissection of the experimental fish, the ethical issues were followed according to Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Initially, morphometric and meristic counts of three individuals of each species were studied in the laboratory to identify the specimens up to the species level following the works of previous authors (Rahman 2005; Froese & Pauly 2011). The total length (TL), standard length (SL) and total body weight (BW) were measured using digital slide calipers and an electronic balance respectively. In each month, 20 samples of each species were preserved in a 10% formalin buffered solution for the investigation of food and feeding ecology.

***Length-weight relationship***. The length-weight relationship is expressed as: $W=aL^b$ and represented linearly by logarithms transformation: Log W = Log a + b Log L (Pauly 1993). Where W and L were fish body weight in gram and total length in centimeter, respectively. Parameters "a" and "b" are the regression coefficients, which were estimated by the least squares regression method. The correlation ($r^2$), that is the degree of association between the length and weight, was computed from the linear regression analysis.

***Condition factor***. The condition factor of the fish was calculated using the formula developed by Ricker (1973):

$$K= W\times100/L^3$$

Where:
K - condition factor;
W - fish weight (g);
L - total length of fish (cm).

***Study of food habit***. The gut of the fish was removed by making a longitudinal incision along the mid-ventral line from the mouth to the anus to expose the visceral organs. The gut was removed carefully by detaching it from other internal organs and fatty tissues. The gut length (GL) was then measured on a graduated measuring board. The stomach was cut off from the gut and scored 0, 25, 50, 75 or 100% according to its fullness as described by Olatunde (1978).

The relative length of gut (RLG) is the most common index which has been widely used to determine the feeding habit of fish. The RLG value varies from species to species and it was measured by using the following formula (Olatunde 1978):

$$\text{RLG (Relative Length of Gut)} = \frac{\text{Length of Gut}}{\text{Length of Body}}$$

Depending on its value, it can be decided the food habit and feeding behavior of fish species: RLG is <1 for carnivores, RLG is mean (2<RLG<3) for omnivores and RLG>2-3 for herbivores.

***Gut content analysis***. The contents were kept into a petri dish which was then observed under a monocular microscope. The food materials were identified with the aid of keys provided by Mellanby (1975). The gut contents were analyzed by frequency of occurrence method as described by Hynes (1950) and Laevastu (1965). Each food item was identified and the number of guts in which the food occurred was counted and expressed as a percentage of the total number of guts as follows:

$$P = (b/a) \times 100$$

Where:
a - total number of fish examined with food in the gut;

b - number of fish containing a particular food item;
P - percentage of occurrence of each food item.

***Statistical analysis***. The regression and correlation analyses were carried out using Microsoft Excel, computed intercept (a), slope (b), correlation coefficient (r), coefficient of determination ($r^2$), standard error of intercept ($se_{lna}$), standard error of slope ($se_b$), confidence interval (CI), RLG.

## Results and discussion

***Length-weight relationship***. A total of 243 specimens of the three species belonging to the genus of *Barilius* were analyzed (Table 1). Sample size, length and weight range, values of intercept (a), slope (b) and coefficient of determination ($r^2$) of the collected individual of each species are shown in Table 1 and Figures 2-4. The lengths ranged 7.0-15.6 cm for *B. bendelisis,* 4.9-7.9 cm for *B. barna* and 6.1 to 10.8 cm for *B. tileo*, while the weight ranged 3.55-40.10 gm, 1.2-5.25 gm and 2.61-11.94 gm for *B. bendelisis*, *B. barna* and *B. tileo*, respectively.

Table 1

Morphometric parameters of *Barilius* spp. from the Atrai river

| *Parameters* | *Name of species* | | |
|---|---|---|---|
| | *B. bendelisis* | *B. barna* | *B. tileo* |
| Sample size | 57 | 102 | 84 |
| Length range (cm) | 7.0-15.6 | 4.9-7.9 | 6.1-10.8 |
| Weight range (gm) | 3.55-40.10 | 1.20-5.25 | 2.61-11.94 |
| a | - 3.8954 | - 4.0717 | - 3.734 |
| b | 2.7782 | 2.7686 | 2.6297 |
| $r^2$ | 0.8693 | 0.8206 | 0.8335 |
| K±SD | 1.209±0.084 | 1.151±0.145 | 1.196±0.184 |

a – intercept; b – slope; $r^2$ - coefficient of determination; K - condition factors; SD - standard deviation.

The generalized ln-ln relationships of total length and body weight of *B. bendelisis, B. barna* and *B. tileo* were $BW=0.020TL^{2.778}$, $BW=0.017TL^{2.768}$ and $BW=0.026TL^{2.629}$, respectively, that showed a positive correlation at 0.05% level of significance. The b values for length-weight relationships estimated in this study were in expected to range 2.5 to 3.5 (Froese 2006). The b values were 2.7782, 2.7686 and 2.6297 for *B. bendelisis, B. barna* and *B. tileo*, respectively, indicating negative allometric growth patterns (Figures 2, 3 and 4). The results of this study were congruent with the results of other studies: b=2.5-3.5 (Xue et al 2011) and b=2.81-3.32 (Gupta et al 2011). The values may vary depending on various factors such as time, season, habitat, gonadal maturity and sex, that ultimately affect the growth of fish (Bagenal & Tesch 1978). In our present study, temporal variations of b value were observed for all species. In case of *B. bendelisis* the values ranged from 2.35 to 3.23 where the minimum 'b' value was found in December and the maximum in January (Figure 2). At 95% confidence limits, the 'b' value revealed that the growth in January was isometric that might be due to high concentration of food consumed in this month whereas the growth was allometric in November, December, February, March and April. Similarly, *B. barna* and *B. tileo* showed an isometric growth in January and April (for *B. barna*, b=2.13-3.20; for *B. tileo,* b=2.19-3.11), and an allometric growth in November, December, February and March and April (Figure 3, Figure 4).

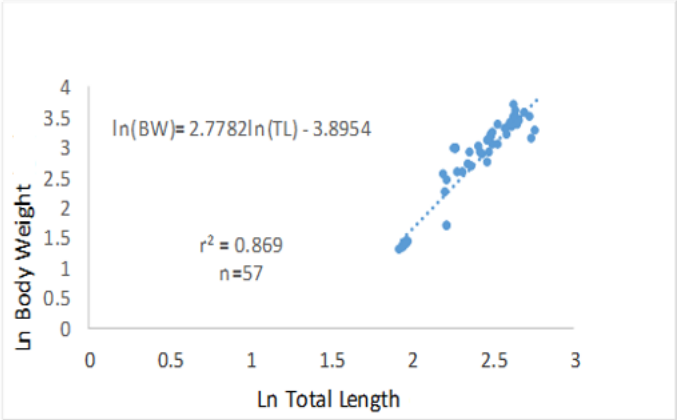


Figure 2. Length-weight relationship of *Barilius bendelisis.*

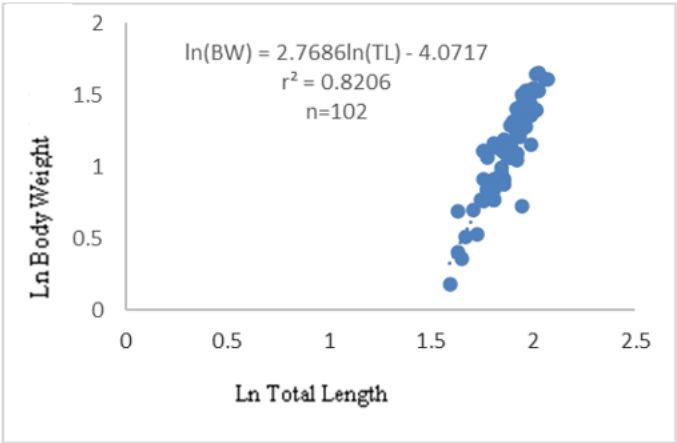


Figure 3. Length-weight relationship of *Barilius barna.*

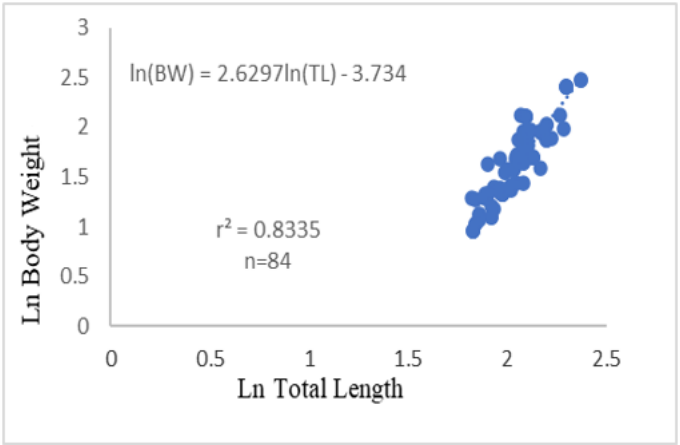


Figure 4. Length-weight relationship of *Barilius tileo.*

***Condition factors***. Condition factor has been used as an indicator of fish health. This factor is calculated from the relationship between the weight of a fish and its length, with the intention of describing the "condition" of that individual fish (Faroese 2006). In this study, the condition factor (K) values were 1.245±0.084 for *B. bendelisis*, 1.201±0.110 for *B. barna* and 1.258±0.249 for *B. bendelisis.* The monthly variations of condition factors (K) for the three species are shown in Figures 5-6. It was observed that all the three species of genus *Barilius* exhibited higher value of condition factors (K) in February. This might be due to the availability of more food in this month. In contrast, the lowest K values were observed in April for *B. bendelisis* (1.182±0.053) and *B. tileo*

(1.089±0.164), while in the case of *B. barna* (1.110±0.226) the lowest level occurred in January (Figure 5, 6, and 7). A similar seasonal variation in the relative condition factor ($K_n$) was reported by Yilmaz et al (2012) while studying the length-weight relationship of white bream (*Blicca bjoerkna*), a fish species belonging to the family Cyprinidae. They observed that the mean values of seasonal $K_n$ for female and male white bream ranged from 0.988 (summer) to 1.028 (spring) and from 0.971 (autumn) to 1.015 (winter), respectively.

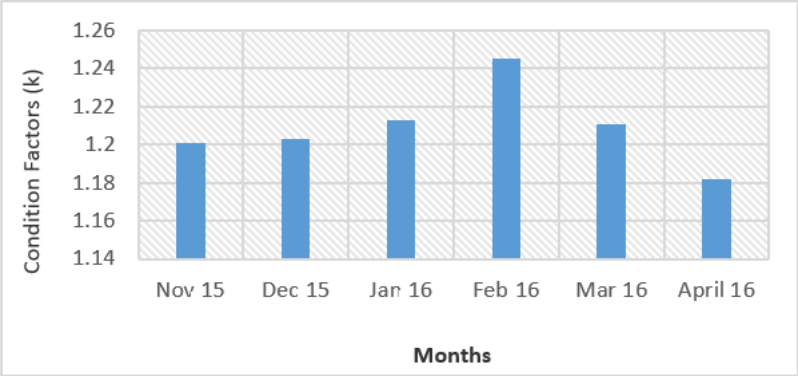


Figure 5. The condition factors of *Barilius bendelisis* from November 2015 to April 2016.

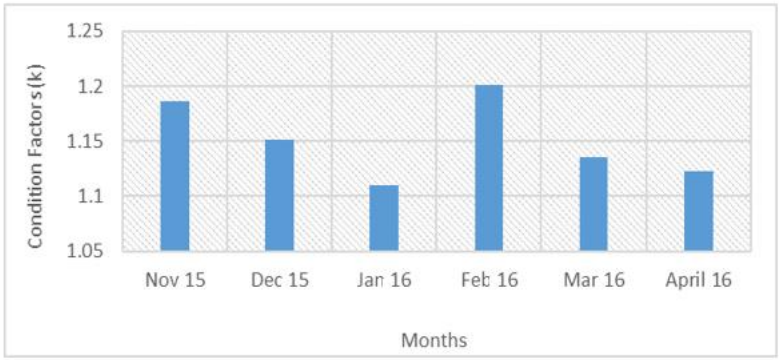


Figure 6. The condition factors of *Barilius barna* from November 2015 to April 2016.

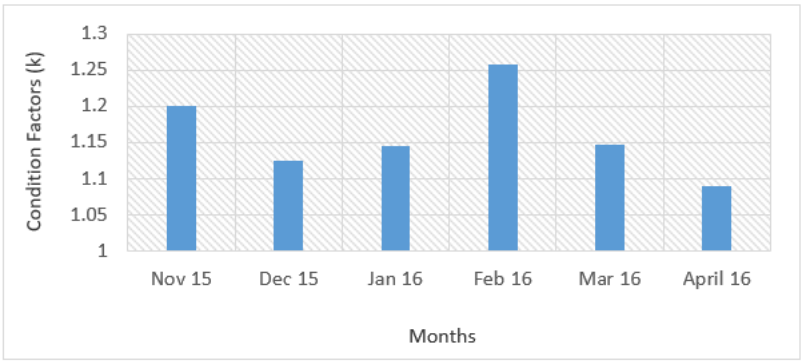


Figure 7. The condition factors of *Barilius tileo* from November 2015 to April 2016.

***Study of food habit***. The results of the gut content analysis of three species of the genus *Barilius* examined for six months were shown in Table 2. The plant food items recorded in the gut content were Bacillariophyceae (*Chlorella*, *Ulothrix),* Chlorophyceae (*Fragillaria)* and Cyanophyceae (*Anabaena*). On the other hand, crustaceans (shrimp) were found dominant among zooplankton item and its frequency of occurrence were recorded 48.57 for *B. bendelisis*, 41.94 for *B. barna* and 62.85 for *B. tileo*. Food habit of fish has a great significance in aquaculture practice. The results of the gut content analysis of three species of the genus *Barilius* suggested that they are omnivore feeders,

as food items identified were of plant and animal origins, which is also confirmed by Malami et al (2004). Substrates like stones and rocks harbor the highest density of bacillariophyceae community in lotic systems, hence observation of higher proportion of bacillariophyceae in the gut of both size groups indicates that *B. bendelisis* browses on stone and rock substrate (Nautiyal 1999) which are congruent with the bottom topography of the Atrai river, Dinajpur.

Table 2

Gut content analysis of *Barilius* spp. by frequency of occurrence methods

| *Food item* | | *Frequency of occurrence methods* | | |
|---|---|---|---|---|
| | | *B. bendelisis* | *B. barna* | *B. tileo* |
| Phytoplankton | *Chlorella* | 28.57 | 25.80 | 28.57 |
| | *Ulothrix* | 05.71 | 9.67 | 08.57 |
| | *Fragillaria* | 17.12 | 22.58 | 05.71 |
| | *Anabaena* | 11.43 | 12.90 | 05.71 |
| Crustacean | Shrimp | 48.57 | 41.94 | 62.85 |
| Others | Sand | 25.71 | 38.71 | 22.85 |
| | Mud | 14.29 | 22.5 | 11.42 |
| | Unidentified food items | 11.43 | 12.90 | 14.28 |

The food habit of three species was also investigated through the observation of gut fullness (Table 3). The highest result showed with a full gut (minimum 50% food content) and with highest proportion of specimens for all three species. The highest percentages were 34.29% for *B. bendelisis,* 35.48% for *B. barna* and 31.43% for *B. tileo.* On the other hand, in empty condition, lowest percentages of fullness were observed 8.57%*,* 12.90% and 8.57% for *B. bendelisis, B. barna and B. tileo* respectively*.* The overall results of gut fullness revealed that only two percent of guts were empty, while various quantities of food items were found in 98% of guts. The occurrence of a higher percentage of non-empty guts may have resulted from the immediate arrest of food digestion through the injection of formalin into the gut region of the fish before its conveyance to the laboratory after capture. This observation was in line with the report of Malami et al (2004) on the same species.

Table 3

Gut fullness of *Barilius* spp*.* from Atrai River

| *Gut fullness* | *Number of B. bendelisis* | *% Fullness* | *Number of B. barna* | *% Fullness* | *Number of B. tileo* | *% Fullness* |
|---|---|---|---|---|---|---|
| 0 (ES) | 3 | 8.57 | 4 | 12.90 | 3 | 8.57 |
| 1\4 | 9 | 25.71 | 6 | 19.35 | 10 | 28.57 |
| 1\2 | 12 | 34.29 | 11 | 35.48 | 11 | 31.43 |
| 3\4 | 5 | 14.29 | 4 | 12.90 | 6 | 17.14 |
| Full | 6 | 17.14 | 6 | 19.34 | 5 | 14.29 |
| Total | 35 | 100 | 31 | 100 | 35 | 100 |

The RLG value in the three species was given in Table 4. The higher mean RLG of *B. bendelensis* (2.01±0.21) was observed in April and the lower (1.57±0.17) in December. Similarly, the higher (1.93±0.123) and lower (1.01±0.012) mean RLG of *B. barna* was found in November and January, respectively. While, *B. tileo* showed a higher mean RLG in April (1.78±0.21) and a lower one in November (1.25±0.180). The mean RLG values for the three *Barilius* spp. indicate that these species were omnivorous. According to Yamagishi (2005), a RLG value of less than 1 indicates that a fish is carnivorous, whereas an RLG greater than 1 indicates that the fish is herbivore or omnivore. The results of the current study corroborate with Dasgupta (2004), who observed that the average RLG value were 0.7 in carnivorous, 4.77 in herbivorous and 1.37 in omnivorous and 3.7 in plankton feeder fish. In similar way Hanjavanit et al (2013) studied the RLG value for the

species *Syncrossus helodes* and *Yasuhikotakia modesta* and observed that the mean RLG for *S. helodes* and *Y. modesta* were lower than 1 for both seasons, which indicates that these species are carnivorous.

Table 4

The relative length of gut of *Barilius* spp. in six months

| *Sampling months* | *B. bendelisis* | *B. barna* | *B. tileo* |
|---|---|---|---|
| | *Mean±SD* | *Mean±SD* | *Mean±SD* |
| Nov 2015 | 1.68±0.212 | 1.93±0.123 | 1.25±0.180 |
| Dec 2015 | 1.57±0.171 | 1.83±0.124 | 1.50±0.109 |
| Jan 2016 | 1.72±0.130 | 1.01±0.012 | 1.38±0.171 |
| Feb 2016 | 1.91±0.192 | 1.19±0.141 | 1.78±0.210 |
| Mar 2016 | 1.85±0.141 | 1.12±0.130 | 1.51±0.120 |
| April 2016 | 2.01±0.210 | 1.34±0.082 | 1.43±0.013 |

**Conclusions**. This study has provided basic information on length-weight relationship, condition factor and food habit of *Barilius*. The study concluded that the length-weight relationship is positively related and indicated negative allometric growth patterns. The condition factors of these species were found to vary in different months depending on different environmental factors. The gut content analysis of three species of the genus *Barilius* suggested that they are omnivore feeders. The results of this study would be an effective tool for fishery biologists, managers and conservationists to initiate early management strategies and regulations for the sustainable conservation of the remaining stocks of those three species in the Atrai River, Dinajpur and surrounding ecosystems. Also, this study would be useful for further studies on the population assessment of the species in Bangladeshi waters and neighboring countries.

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**Conflict of interest**. The authors declare no conflicts of interest.

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Authors:
Mosammat Iffat-Ara, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: mstiffatara931@gmail.com
Rubaiya Pervin, Department of Fisheries Management, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: rubaiya2015@hstu.ac.bd
Nipa Gupta, Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: nipagupta@hstu.ac.bd
Mosammat Boby Akter, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: bobyhstu28@gmail.com
Sharmin Ahmed, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: sharminahmed1230@gmail.com
Zubaida Parveen Patwary, Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: rini.zubaida@gmail.com
Imran Parvez, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, e-mail: iparvez.fbg@hstu.ac.bd

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