Biology of the endangered queen loach (*Botia dario)* collected from wild sources in Bangladesh

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Abstract. The study was conducted with the aim of evaluating the food and feeding habit of *Botia dario*, collected from wild sources of north-eastern Bangladesh from January to June 2019, by calculating the: Gastrosomatic Index (GaSI), Relative Gut Length (RGL), Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI). Food and feeding habit were evaluated based on the gut content analysis followed by the percent of numerical method and the frequency of occurrence method. Observation of feeding habit of *Botia dario* revealed that it is a carni-omnivorous and bottom feeder fish having a preference for animal materials (89.06%) over plant materials (2.67%). By percent of numerical method, the main contributors to their diet were worms (46.40%), followed by fish particles (28.80%), crustaceans (5.60%), insects (5.33%), detritus (3.20%), molluscs (2.40%), algae (1.87%), plant parts (0.80%) and water mites (0.53%). By frequency of occurrence method, worms and fish particles had a similar contribution (94.44%) to *B. dario* diet, followed by insects (66.67%), crustaceans (55.56%), algae (38.49%), detritus (33.33%), molluscs (22.22%), plant parts (16.67%) and water mites (11.11%). Average RGL value of *B. dario* was 1.08±0.16 which also demonstrates the carni-omnivorous nature of the species. Maximum GSI value was found in the month April (11.29±1.53), and HSI value was lowest in April for both female (1.69±0.77) and male (1.85±0.18). The highest GSI value and lowest HSI values in April indicate that it is the spawning period for this species, because the liver has a weight loss during reproduction which may imply the mobilization of the hepatic reserve for the maturation of gonads. Moreover, the lower RGL (0.98±0.03) and GaSI (0.91±0.56) values in April indirectly confirmed that April is the spawning period for *B. dario*. The findings might be useful as baseline information on the biological characteristics of *B. dario*.
Key Words: food and feeding habit, GaSI, GSI, HSI, carni-omnivorous, biological characteristics.

Introduction. *Botia dario* (Hamilton 1822), also known as Queen loach or Bengal loach or Necktie loach, has yellow golden stripes on a black background. The species is one of the most active loaches living in South East Asian countries including Bangladesh, India, Bhutan and Nepal (Siddiqui 2007). It is one of those few Small Indigenous Species (SIS) having both edible as well as ornamental values (Dey et al 2015). In Bangladesh, it is regarded mainly as a table fish due to its excellent flesh quality (Hussain et al 2007) with remarkably higher amount of fat and minerals content (calcium, phosphorus etc.), as compared with large freshwater fishes (Hossain et al 1999). A moderate demand for this species originates among the aquarium fish hobbyists due to its brilliant color pattern (Gupta & Banerjee 2012). *B. dario* also started being exported to different countries (Gupta & Banerjee 2014). Although this fish species was previously abundant in the rivers, streams and beels (seasonal low-lying floodplain) throughout Bangladesh, serious declines in its populations and abundances have been recently reported (Hossain et al 2015). It is inferred that the natural population of *B. dario* declined by about 60% over the last 20 years (IUCN 2015) due to a number of factors like: habitat loss resulting from the use of insecticides in paddy fields, siltation of upland rivers, lifting of stones and sands from river beds and construction of flood control dams, ecological changes, over exploitation, destruction of breeding ground and lack of proper management (IUCN 2015;

Hossain et al 2015). A drastic decline of the population in in Bangladesh natural water bodies has categorized the species as endangered (IUCN 2015).

Therefore, appropriate conservation actions are urgent to save the species. Two main conservation strategies might be: to protect a fish species it in its natural habitat and/or to bring it to aquaculture purview. In Bangladesh, *B. dario* can be conserved in its natural habitat by banning of fishing during spawning season to provide protection to the brooders (Hossain & Alam 2015) and by establishing suitable sanctuaries in preferred areas of natural water bodies (Hossain et al 2009), which ultimately helps to stock the re-population of the species. However, prior to make any conservation strategy and for its effective implementations, biology of the species needs to be studied thoroughly. Unfortunately, there's scanty of existing literature on different biological aspects of the species. Very few researchers work on different *B. dario* biology topics, including length-weight relationship (Haque & Biswas 2014a; Das & Bordoloi 2015), reproductive biology (Dey et al 2015; Hussain et al 2007; Hossen et al 2014; Haque & Biswas 2014b; Das & Bordoloi 2015), morphometry (Haque & Biswas 2014a), proximate composition and nutritional value (Jena et al 2018; Hossain et al 1999), etc. There are no considerable documents available on food and feeding habit (Gupta 2016) and in particular on the HSI (Hepatosomatic Index) of the species. Besides, information on the reproductive biology is not sufficient because of variation in its breeding season (Gupta 2016), therefore, further research is worthwhile on reproductive biology to fill up the existing information gap.

Thorough knowledge on the food and feeding habit, essentially the stomach content analysis with Gastrosomatic Index (GaSI) and the Relative Gut Length (RGL), is essential for rearing and culture of any fish species (Gupta 2016). Stomach content analysis provides important insights into fish food patterns and a quantitative assessment of food habits is an important aspect of the fisheries management. GaSI has been used to determine the seasonal feeding intensity and sometimes indirectly indicates the spawning season in certain species of fish (Alam et al 2016; Dadzie et al 2000). RGL has been extensively used to evaluate the feeding habits of aquatic species (herbivorous, carnivorous, omnivorous, herbi-omnivorous or carni-omnivorous) (Das & Moitra 1963; Dasgupta 2004). Gonadosomatic Index (GSI) along with Hepatosomatic Index (HSI) are used to assess the reproductive season and condition of fish (Arruda et al 1993). GSI value of different fishe species has been considered by many researchers for determining spawning season and spawning frequency (Islam et al 2008; Ghaffari et al 2011; Kingdom & Allison 2011; Jan et al 2014). Liver produces vitellogenin (the yolk precursor) and plays significant roles for the development of ova, which inspires many researchers to work with HSI (Jan & Jan 2017). Besides, HSI describes the status of energy stored in fish and is considered as a good indicator of the recent feeding activity of the species (Tyler & Dunns 1976). Therefore, the present study was conducted to explore the food and feeding habits, the hepatosomatic index and the spawning season of *B. dario*, which may subvene to some extent to the existing knowledge gap and also to help adopting a conservation approach to this natural resource.

Material and Method

A total of sixty-four fish samples were collected (10-12 individuals, in the last week of each month) from natural waters of the North-Eastern region of Bangladesh using seine nets, with the help of local fisherman, over six months of laboratorial research. Three sampling locations were: Mohashing River in Sunamganj district (24°55'52.5"N; 91°26'54.3"E); Kushiyara River in Sylhet district (24°39'11.6"N; 91°49'36.9"E); Kushiyara River in Moulovibazar district (24°39'12.0"N; 91°49'51.7"E). Immediately after catch, fish specimens were preserved in 10% formalin solution and transported to the laboratory for further analysis.Total length ofl fish specimens was measured with a digital slide calipers having ±0.01 mm accuracy (EAGems-B00Z5KETD4) and weighted with a digital balance having ±0.01 g accuracy (Shimadzu UX320G). Then the fish specimens were dissected out and all internal organs were removed by using a soft brush and a blunt forceps to avoid any accidental injury or cut. Alimentary tract, gonad, and liver were detached and preserved in 10% formalin solution, in leveled vials. Alimentary tract length and pharynx length were measured by digital slide calipers. Then the stomach was detached from the alimentary tract. Stomach, liver, and gonad were weighted with a digital balance.

The intensity of feeding was judged by the degree of distension of the stomach or by the quantity of food that it contained. The distension of the stomach was classified as 'full', '3/4 full', '1/2 full', '1/4 full' and 'empty' by eye estimation following Nagar & Sharma (2016). Further, the stomachs were dissected and 1mL of food contents were collected in a glass vial and identified under a light microscope (XSP L101), in order to determine different food items ingested by the fish. The food items encountered were analyzed using the numerical count method (Hynes 1950) and the frequency of occurrence method (Hynes 1950) using the following equations.

$$\text{Percentage of numerical count} = \frac{\text{Number of individual food items}}{\text{Total number of food items}} \times 100$$

$$\text{Percentage of occurrence of a food type} = \frac{\text{Number of guts where the food occurred}}{\text{Total number of guts analyzed}} \times 100$$

The month wise feeding intensity or Gastrosomatic Index (GaSI) was calculated after Desai (1970) using the formula:

$$\text{GaSI} = \frac{\text{Weight of the stomach}}{\text{Weight of fish}} \times 100$$

The Relative Gut Length (RGL) was calculated using the following formula (Al-Hussaini 1949).

$$\text{RLG} = \frac{\text{Length of gut}}{\text{Total body length}}$$

Only female specimens were studied to determine the Gonadosomatic Index (GSI). Females were separated from males by examining their gonad. Large-sized ovary was identified by eye examination. For identifying tiny-sized gonad, aceto-carmine was applied for staining, then the sample was examined under a light microscope to enhance the contrast and for a clear visualization of ovum (Wassermann & Afonso 2002). If small-sized ova were seen under the microscope, it gives confirmation that it was ovary. GSI was calculated by the formula of Afonso-Dias et al (2005).

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

HSI of female fish and male fish was studied separately in order to relate with GSI of female. HSI was calculated according to the formula of Rajguru (1992).

$$\text{HSI} = \frac{\text{Weight of liver}}{\text{Weight of fish}} \times 100$$

All data were analyzed using Microsoft Excel® (2010) and relevant graphs and tables were prepared. Data were presented as arithmetic mean±standard deviation if otherwise not mentioned.

Results. Feeding intensity was observed on the basis of eye estimation and stomach distention. Over the examined stomachs, 28.13% were full, 26.56% were ¾ full, 23.44% were ½ full and 21.88% were ¼ full and none was empty. Specimens with full, ¾ full and ½ full stomachs were considered to feed actively and specimens with ¼ full stomachs were considered to feed inactively. The overall percentage occurrence revealed that 78.13% of the fish samples showed active feeding during the study period. The percentage value of stomach contents indicates that fish samples mainly fed on worms (46.40%), fish particles (28.80%), crustaceans (5.60%) and insects (5.33%) (Table 1). It also shows that diet of *B. dario* was mainly composed of 89.06% animal materials and 2.67% plant materials, suggesting it is a carni-omnivore species (Table 1). The values of occurrence frequency were: 94.44% for worms, 94.44% for fish (and 66.67% for insects (Table 1).

Table 1

Food composition of *Botia dario* on the basis of percentage numerical count and percentage occurrence frequency method

Food materials	*Food items*	*% of numerical count*	*% of occurrence frequency*
Animal materials (89.06%)	Worms	46.40	94.44
	Fish particles	28.80	94.44
	Crustaceans	5.60	55.56
	Insects	5.33	66.67
	Molluscs	2.40	22.22
	Water mites	0.53	11.11
Plant materials (2.67%)	Algae	1.87	38.49
	Plant parts	0.80	16.67
Miscellaneous (8.27%)	Detritus	3.20	33.33
	Unidentified	5.07	

GaSI helps to determine stomach fullness and feeding condition of the species. The GaSI showed monthly variations between the peak in March (2.43±1.62) and the lowest level in April (0.91±0.56) (Figure 1).

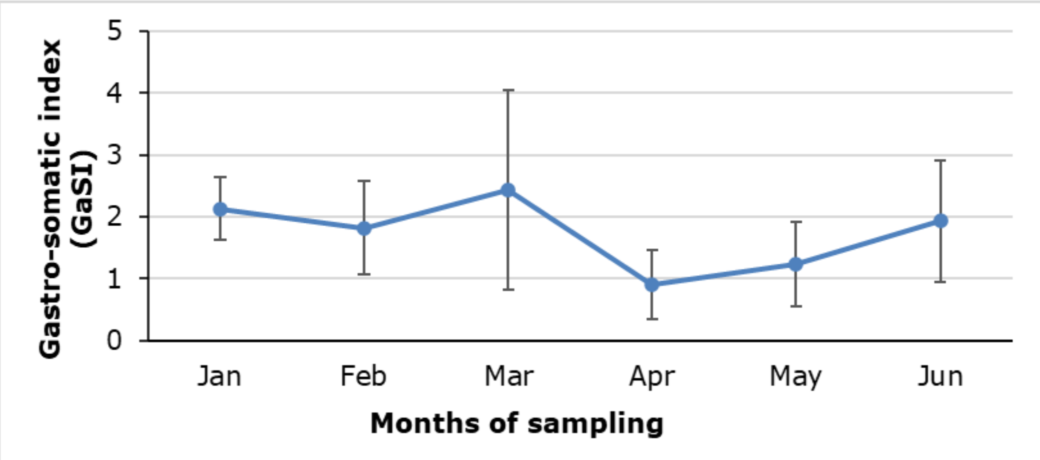

Figure 1. Changes of gastro-somatic index in *Botia dario.*

Table 2

Total length, gut length and RGL value of *Botia dario* in different months

Month	*Total length (cm)*	*Gut length (cm)*	*RGL*
January	8.95±1.92	9.72±2.55	1.09±0.19
February	8.99±1.29	9.17±1.73	1.02±0.07
March	10.76±1.99	12.84±4.01	1.19±0.26
April	9.48±1.06	9.31±0.92	0.98±0.03
May	8.42±0.83	9.38±1.72	1.11±0.14
June	8.48±1.02	9.72±1.53	1.15±0.11

Relative gut length helps to determine the feeding habit of a species and it has also a definite relationship with length and life stage of fish. In the present study, minimum and maximum body lengths were 6.6 cm and 12.9 cm, respectively, with an average of 9.08±1.52 cm. The gut length of *B. dario* varied from 4.9 cm to 19.1 cm, with an average of 9.88±2.38 cm. Average RGL value was 1.08±0.16, which indicates this is a carni-omnivore in nature. Highest RGL value was recorded in the month of March (1.19±0.26) and the lowest in April (0.98±0.03) (Table 2).

Only female fishes (68.75%) were subjected to determination of GSI. HSI of female and male fish was calculated separately in order to relate with the GSI of females. The GSI value was increased gradually from January to April. The maximum value of GSI was recorded in April (11.29±1.53) and the minimum in January (1.05±1.33) (Figure 2). After April, GSI value started to decrease gradually. This suggests that *B. dario* reached a breeding condition peak in the month of April. Also, a majority of males were found in oozing condition, bright in color during the same month. The HSI values of males increased gradually from January to March, but it was the lowest in April (1.85±0.18) and the highest in June (5.19±2.67) (Figure 2). The HSI value of female fish increased gradually from January to February and slightly decreased in March, but it was lowest in April (1.69±0.77) and the highest in May (3.94±1.72) (Figure 2). Thus, a low hepatic activity was found during the month of April for both males and females, which also suggests that April is the breeding month of *B. dario*.

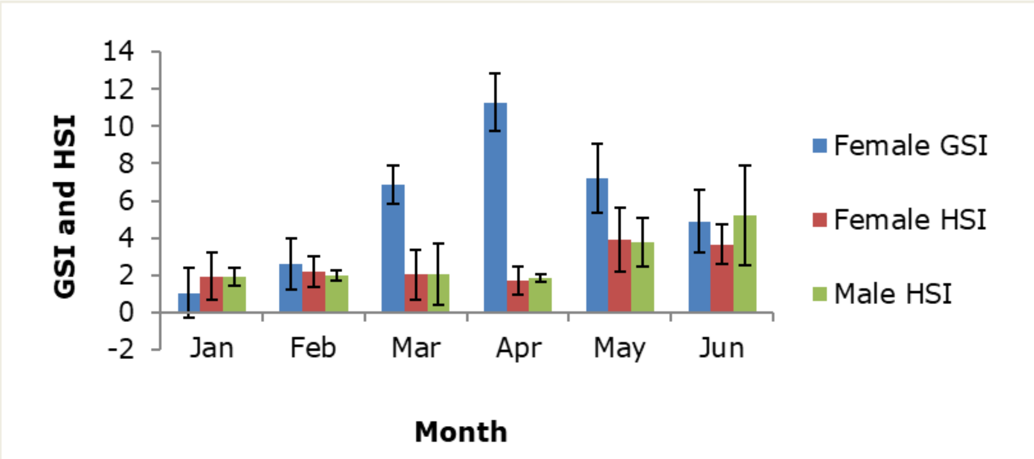

Figure 2. Month wise GSI value for female and HSI value for females and males.

Discussion. *B. dario* has a significant role as a food fish as well as an ornamental fish in the South Asian countries, including Bangladesh. The species is marked as threatened in Bangladesh (IUCN 2015) and its wild population is declining due to overfishing, habitat degradation and ecological changes (Hossain et al 2015). Previous studies on the species biology, especially on feeding and breeding, are very scarce (Gupta 2016), which made the species one of the least investigated in the region. The current study is the first attempt to investigate on the food and feeding habit of this species. The assessment of overall feeding intensity revealed that none of the stomachs of sample fish specimens was empty and most of the fish (78.13%) fed actively during the study period; this is probably an indication of both the efficiency of the sampling method adopted in this study and of the healthy feeding habit of this species, that allows them to use accessible food items in their habitat. Similar results were found by Olele (2011) and Nwani (2004), demonstrating that a large number of guts with food were found due to feeding strategy, food availability and efficiency of sampling method.

Survival, growth and reproductive activities of a species highly depend on food and feeding habit. Stomach content analysis showed that the food items of *B. dario* consists mainly of worms (46.40%), fish particles (28.80%), detritus (3.20%), algae (1.87%) and a small amount of crustaceans and plant parts. By diet frequency of occurrence method, worm and fish particles have similar contributions (94.44%), followed by insects (66.67%) and crustaceans (55.56%). These findings revealed the fact that worms, fish particles and insects were dominant in the diet composition of *B. dario*. The animal food formed 89.06% and the plant food formed 2.67% of the total diet composition, demonstrating that, although this fish has a preference for animal diet, it also eats vegetative matters (soft leaf aquatic plant, algae, plant parts etc.). Their diet covers a broader spectrum of food ranging from various types of worms, fish, insects, crustaceans, molluscs, algae, plants and detritus which indicates that the fish has a carni-omnivorous nature. Nagar & Sharma (2016) also reported the carni-omnivorus nature of a species named *Noemachielus botia* from the *Cobitidae* family, based on their diet composition, revealing: 65.3% animal origin and 19.9% plant origin. In the same study, the highest percentages in the diet were reported for microcrustaceans (46.5%), followed by insects larvae (18.8%) and algal matter (12.3%). Agrawal & Tyagi (1969) reported the carnivorous feeding habits of two *Cobitidae* family fish species, namely *Noemacheilus botia* and *Botia lohachata*, which feed mainly on copepods, small crustaceans and insect larvae.

RGL value is often used to determine the feed preferences and also used as a predictor of the general dietary types in a number of fish species (Koundal et al 2013; Osman et al 2019; Berumen et al 2011; Rahman et al 2012). In the present study, average RGL value was found 1.08±0.16 and this result is tentatively expected because it correlates with the feeding habits (a wide range of animal and plant materials). Carnivorous species commonly have a short and simple gut, omnivorous have a moderate gut and herbivorous have a long and complex gut (Al-Hussaini 1949; Elliott & Bellwood 2003). According to Al-Hussaini (1949), an RGL value of 0.5-2.4 indicates the species as carnivore, between 0.8-4.00 as omnivore and higher than 2.00 as herbivore. Thus, the carni-omnivorus nature is also demonstrated by the average RGL value. Month wise observation of Relative Gut Length revealed that the lowest value was found in April (0.98±0.03). After April, the RGL value gradually increased again. The low RGL value occurred in April might be associated with the spawning season of the species. Evidence shows that in the spawning period, the RGL value declined due to gonads development, because most of the body cavity is filled by the ovary during its maturation (Koundal et al 2013; Joadder 2006). It is well documented that food and feeding habits of the fish are largely dependent on the: food availability in nature, environmental conditions, size or sexual stages of fish as well as inter and intra specific competition (Manko 2016). It is also reported that gut length may increase or decrease depending upon the available food types in the surrounding environment and nature of food intake; for example, gut length increased due to more intake of vegetative matter and decreased with the increment of animal matter (Dasgupta 2004). In this case, the nature of *B. dario* is carni-omnivorus as it prefers animal matters to the plant matters.

Food material type has been studied in different fish species for determining the feeding region (i.e. bottom feeder, pelagic feeder). Bottom feeder fish consume a considerable amount of bottom drifting components, including: worms, insects, crustaceans, molluscs and detritus (Nagar & Sharma 2016; Alikunhi 1952, 1957; Khabade 2015). In the diet composition of pelagic fish, a considerable amount of phytoplankton can be found (Hoque et al 2016; Hasan et al 2016; Lalit et al 2015; Costalago et al 2012; Shalloof & Khalifa 2009). From this point of view *B. dario* is a bottom feeder. Moreover, the absence of phytoplankton in the stomach of the species also demonstrates that the species may live on the bottom of the water.

The GaSI depicts the intensity or rate of feeding in various months. A high feeding intensity was observed during March, when the GaSI was 2.43±1.62 and most of the stomachs were full, containing a considerable amount of food. The feeding intensity was generally low during April (GaSI 0.91±0.56), when stomachs contained a low amount of food, which was associated with the peak of the gonads maturity period: during the gonads development, most of fish are affected by a starvation period. The occurrence of low feeding in other fish species, during their breeding period peak, has been reported by several authors (Lanthaimeilu & Bhattacharjee 2018; Alam et al 2016; Dadzie et al 2000; Saikia et al 2015; Shallof & Khalifa 2009; Rao et al 1998; Hatikakoty & Biswas 2003). The highest GaSI value during March might be associated with feeding in pre-spawning period. A considerable increase in the rate of feeding intensity in pre spawning period has already been reported (Mushahida-Al-Noor et al 2013; Bahri-Shabanipour & Mohammadizadeh 2010).

In the present study, GSI value was found maximum in April (11.29±1.53), suggesting that April is the peak period for spawning of *B. dario* in natural habitats.

January to March was the vitellogenin period or active cum quiescent period. In the current study, the GSI value started to decrease gradually after April, whereas this finding doesn't commensurate with the finding of Dey et al (2015), who reported that GSI of *B. dario* increased from April to July. Haque & Biswas (2014b) have also reported that the breeding season of the species lies between March to August, while Das & Bordoloi (2015) reported that its peak is situated between May and August. This variability might be linked to the environmental parameters like: temperature, rainfall, available nutrients in the habitats where fish were collected. Besides, the onset of monsoon period is continuously changing due to the climate change effects (Seth et al 2019), In Bangladesh, nearly all of the freshwater species breed during monsoon, like: *Channa marulius* (Yadav et al 2016), *Mystus gulio* (Islam et al 2008), *Labeo calbasu* (Kabir & Quddus 2013), *Amblypharyngodon mola* (Hoque & Rahman 2008). However, GSI is a vital parameter which provides significant information about the cyclic changes during different seasons. The seasonal timing of the reproduction and spawning period can also be easily identified by the GSI change, which is widely used to determine the reproductive features of a fish species.

In this study, the monthly variation of GSI was related to HSI value. Both females' and males' HSIs were the lowest in April, while their GSI values were the highest. Higher GSI values coupled with lower HSI values might be associated with the mobilization of hepatic reserves for the maturation of gonads (Zin et al 2011). The finding is similar to the results of Sudarshan & Kulkarani (2013), Jan & Jan (2017), who stated that the HSI gradually decreased along with the ovarian maturation. But the present study's findings are in a high contrast with the findings of Hismayasari et al (2015), Allison (2011), Oso et al (2011) and Cek et al (2001), who reported a positive correlation between the GSI and his trends, meaning the HSI values increased along with the GSI.

Conclusions. *B. dario* is a carni-omnivorous fish. April is the peak period for spawning in the natural habitats in Bangladesh. *B. dario* has a decreased RGL value in the spawning season, probably due to rapidly increasing gonad filling its abdominal space. Its GaSI value also decreased in the spawning season, due to the starvation during the development of gonads. Its HSI value decreased in spawning season, due to the mobilization of the hepatic reserves for the maturation of gonads. This research has some limitations: the GSI data were not taken throughout the full year due to a very low availability of the species during the rest the months. Therefore, it is encouraged to work also with the GSI values for six remaining months, which will allow concluding whether the species is a prolific breeder or not.

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