Advances in Bioresearch
Adv. Biores., Vol 10 (2) March 2019: 68-81
©2019 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.10.2.6881

Advances in Bioresearch

ORIGINAL ARTICLE

Reproductive Biology of Flying barb, Darkina, *Esomus danricus* with some related characteristics

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ABSTRACT
The aim of this study was to evaluate the biology of a small fish, darkina, Esomus danricus collected from natural pond located in the Fisheries Field Laboratory complex, BAU, Mymensingh. The experiment was undertaken in a pond using thirty six small hapas (0.25 m^3) and one big hapa (1 m^3). All the hapas were placed in a big pond (420 m^2). The stocking density was 10 sp./hapa and 40 sp./hapa in small (H_s) and big hapa (H_b), respectively. The fishes of three small hapas out of thirty six were emptied and measured with their length (cm) and weight (g) in each month. But the fishes of big hapa were counted and weighed in every month and released in that hapa up to the end. No supplementary feeding was applied for darkina. This experiment was continued from May'13 to April'14. The key biological characteristics i.e. gonado-somatic index (GSI), condition factor (K) and four polygon of ova diameter were given an indication the breeding season extended from June to October. Fecundity and gonad weight was positive correlation which was expressed as the equation, Y= 5705.x + 1926 (R^2=0.605). Furthermore, to get increased or decreased number of fishes could presumably the spawning period. Within the suitable physico-chemical parameters, monthly morpho-histological examination of the gonad disclosed the existence of five maturity stages (immature, maturing, mature, ripe and spent & resting) in darkina. Both the male and female were represented more or less similarly breeding periodicity. Considering microscopic and histological assessment of fishes occur the peak breeding in June & September. The experimental fish was also established to be a manifold spawner. The knowledge of key characteristics would be useful to impose adequate regulation for the conservation and proper management of the species.
Keywords: ***Esomus danricus,*** *gonado-somatic index (GSI), condition factor (K), fecundity estimation*

Received 05.01.2019 Revised 30.01.2019 Accepted 01.03.2019

How to cite this article:
S Mondal, Md. A Wahab, B K Barman and Md. M M Hossain. Reproductive Biology of Flying barb, Darkina, *Esomus danricus* with some related characteristics. Adv. Biores. Vol 10 [2] March 2019: 68-81

INTRODUCTION

The flying barb Darkina, *Esomus danricus* is commonly known as darkina, danrika, darka, dadhika etc in Bangladesh local area. It is laterally compressed body, mouth small, directed obliquely upward with a fleshy lower lip. Lateral line incomplete, ceasing after 4 or 5 scales or may be absent. Dorsal fin placed in the posterior half of the body. Anal origin below posterior base of the dorsal. Pelvic equidistant from the snout over the pelvic, outer ray of pelvic elongated. A broad black lateral band from the eye to the caudal base. Margin of the scales of the upper half of the body dotted. Yellowish-white beneath [42]

The fish has the habit of jumping above the water surface. Usually the fish catches with other minnows in ditches, ponds and beels during the dry season. Found in small stream, ponds weedy ditches beels and inundated fields. This fish is widely spread in Bangladesh, India, Pakistan, Nepal, Afganistan, Srilanka and Mayanmar and abundant in rainy season. It is largely preyed upon by snakehead and controls the aquatic insect population and algal bloom in the surface layer of the aquatic environment. By eating aquatic detritus they keep the water clean.

With a view to fisheries management, since the managers need to know the real number spawning fish in a population to be able to manage fishing effort effectively [12]. Determining the reproductive potential of fish populations and monitoring of changes in biological characteristics of exploited fish stock [54]. Estimating the reproductive period and length of gonadal maturation will assist to allow for accurate implementation of fishery legislation [20]. So, the validation of gonad maturation stages with histology is also used to determine the spawning pattern of a fish, as well as the types of methods necessary for the determination of annual fecundity [33] .

'Darkina', a small fish, is considered to be a valuable source of vitamin A as it contains about 500-1500 IU (RAE/100 g clean parts). This fish consists of higher amount of minerals per 100 g, Ca - 891 mg, Ca^b -775 mg, Fe -12 mg and Zn - 4 mg [45]. From nutritional point of view, it is as important as mola. It has low market value compared with mola, but suitable for culture in rice fields. The poor people can harvest or buy a bulk of darkina fish to minimize their daily essential nutrients. It is considered as endangered species in Bangladesh. In order to meet up the increased demand of darkina for the poorer people, this fish should be cultured in the ponds or pond adjacent rice-fields. Darkina can be produced through stock enhancement in the wetland systems as well. However, in making efforts of its mass production, it is essential to know its biology (both feeding and breeding) and culture potentials.

MATERIAL AND METHODS

Study area and design of the experiment

The experiment was undertaken in a pond of Fisheries Field Laboratory, BAU using thirty six small hapas (0.5m X 0.5m X 1m=0.25 m^3) and 1 big hapa (1m X 1m X 1m = 1 m^3). All the hapas were placed in a big pond (28m x 15m= 10.5 dec.) (Fig. 1). That pond was in a polyculture system previously. All hapas arranged in a straight line with three rows and each having 12 in numbers. And the big one was another line as well. The inter-connecting rows space was in two (2) meters.

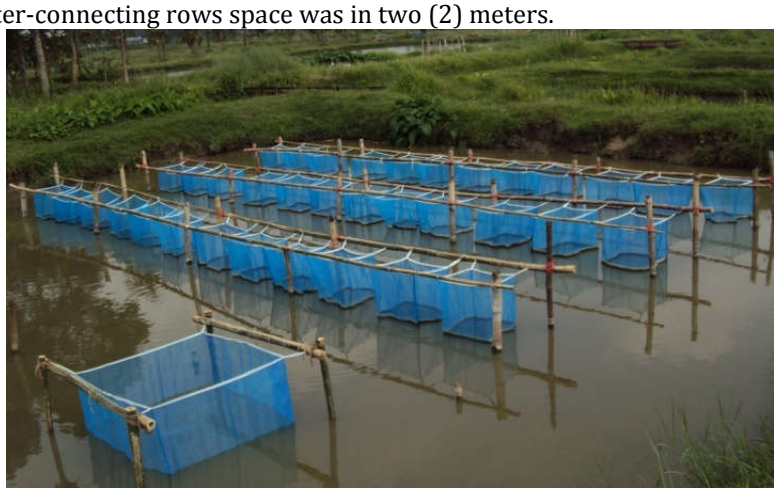

Fig 1. Experimental hapa in the pond

The distance of between two hapas is usually maintained by 0.25 m. The alternate row of hapas would provide enough spacing for easy entrance of air flow and sunlight to ensure congenial environment. The stocking density was 10 sp./hapa and 40 sp./hapa in small (H_s) and big hapa (H_b), respectively. In every month one small hapa of each row was sampled and emptied. No supplementary feeding was applied for darkina. This experiment was continued from May'13 to April'14.

Water quality monitoring

Throughout the experimental period, the water quality parameters were determined once in a month. For water quality measurement, samples were collected between 08:00 to 10:00 AM from sampling hapa and pond water in every month. Transparency measured by a Secchi disc with 20 cm diameter in the pond only. Water temperature was recorded with a Celsius thermometer. DO & pH of water samples were measured by a Portable Multiparameter (HACH). Alkalinity of water was measured by the titration method with the help of 0.02N H_2SO_4 and methyl orange solution. The concentration of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate-phosphorus (PO_4-P) was determined by HACH kit (DR-2010, a direct reading spectrophotometer). Chlorophyll *a* was measured by spectrophotometer (Spectronic GENESYS 5) from the Central Laboratory of BAU [2]. All data were analyzed using for windows (version 20: SPSS Inc, Chicago, USA). One way analysis of variance (ANOVA) was performed and the mean values compared using Tukey's test as post-hoc test.

Plankton enumeration

Ten liters of water samples were collected from five different places of pond and passed through a plankton net (mesh size 20 µm). The concentrated samples were transferred to a measuring cylinder and made up to 50 ml with distilled water. Samples were preserved in small plastic bottles with 10% buffered formalin. Plankton numbers were estimated using a Sedgewick-Rafter counting cell (S-R cell) under a binocular microscope (Olympus, M-4000D) following APHA [2]. Identification of plankton to genus level was carried out using the keys from Ward and Whipple [52], Prescott [40] and Bellinger [8]. The quantitative estimation was done following the same procedure mentioned in Azim *et al*., [4] and the estimation was made by using the following formula:

$N = (P \times C \times 100)/L$

In which,

N= Number of plankton cells or units per liter of original water

P = Total number of plankton counted in 10 fields

C= Volume of final concentrated sample (ml)

L= Volume of original water (l)

Sampling of Darkina

The fishes of three small hapas out of thirty six were emptied and measured with their length (cm) and weight (g) in each month (scale & fine electric balance, College B2002-S). But the fishes of big hapa were counted and weighed in every month and released in that hapa up to end of the experiment. During sampling, big hapa was cleaned by soft brushes for the removal of algae which sometimes build blocks in the meshes of hapa. All the collected fishes were determined by macroscopic and microscopic observation.

Sex determination and fecundity estimation

During the breeding season both male and female were distinguished easily. Although in the early stages, it was difficult to detach them from each other. For breeding purposes using some key characteristics were helpful to separate male and female accurately. The color of mature females was bright, larger in size and very clear dotted on the margin of the scales of the upper half of the body. Female abdomen was soft and swollen, pelvic fins were smooth. Mature females with bloated abdomen had been easily identifiable during the spawning season. At least 5 to 10 fishes were recorded both male and female for the determination of reproductive pattern in each month. Before weighing, the specimen was washed with water and left exposed to air and the excess of moisture was dried off with the help of a blotting paper. Generally more than 1g weighed fishes were selected for gonad separation. Because it was awfully complicated to indentify sex below weight 1g of fish. Without more ado, the sampled fishes were bought to the laboratory and length & weight of each fishes measured. Then gonad of fishes was taken out very carefully and weighing by sophisticated electric balance (PG 503-S Delta Range, Max 510g d=0.01/0.001g METTLER TOLEDO). General facet and configuration as well as month-wise size, shape and color of gonads of the experimental fish were recorded immediately with the help of naked eye and electronic microscope. Assessment of various maturity stages was based on the modified classification of Kesteven [24] and Crossland [13]. Then the sample of gonad was preserved individually in 10% formalin with well labeled for histological examination.

For fecundity estimation the paired ovary of the individual fish was removed and carefully placed on a petridish from female fishes. Ovary was washed and cleaned with distilled water. Weight of the ovary was taken by using electronic balance. Ovary was then also preserved in 10% formalin for fecundity estimation. Male and female gonads have been grouped into different gonadal stages of development by using macroscopic and microscopic observation according to Nikolsky [38], Azadi and Mamun [3] and LeCren [29]. Spawning periodicity has been determined by monthly evaluation of the gonado-somatic index and condition factor. Gonado Somatic Index (GSI) and Condition Factor (K) have been measured using the following formulae:

$$\text{Gonado-somatic index (GSI)} = \text{Gonad Weight (g)} \times 100 / \text{Total body weight (g)} \;\&$$

$$\text{Condition factor (K)} = W \times (10)^5 / L^3,$$

where W is body weight (g) and L is body length(mm)

Among the three fecundity estimation methods like Volumetric, Gravimetric & Von Vayer [28], last one is more possibility to minimize error due to its simple and easy sampling technique. Considering large amount & narrow ova dia, the gravimetric or weight method has been employed in the present study, for its greater efficacy over the other methods. Some other researchers, Phillips [39], Rao [44], Mustafa and Ansari [36] and Shafi and Quddus [47] were applied that method successfully. Using the help of ocular

and stage micrometer, the measurement of ova dia-meter and their size frequency of intra-ovarian oocytes distribution at different times of the months in a year was a common method [30] in determining the maturity cycle of the fish.

Histology of gonad

The previously preserved gonads were taken out in perforated plastic holder, which covered by perforated steel plates. Cleaning, infiltration and dehydration process has been carried out in an automatic tissue processor using a series of alcohol of increasing concentrations, two changes of xylene and finally through molten wax (three series) as mentioned in Table 1.

Table 1. Time schedule in the automatic tissue processor

Sl. No.	Step of process	Time (hour)	Process
1	50% Methylated spirit	1	
2	80% Methylated spirit	2	
3	100% Methylated spirit	2	
4	100% Methylated spirit	2	Dehydration
5	100% Methylated spirit	2	
6	100% Alcohol	2	
7	100% Alcohol	2	
8	Xylene	2	
9	Xylene	1	Clearing
10	Molten wax	1	
11	Molten wax	2	Infiltration
12	Molten wax	2	

Table 2. Staining procedure

Sl. No.	Solution	Times (min.)	Process
1	Xylene	2	
2	Xylene	2	Clearing
3	100% Alcohol	2	
4	100% Alcohol	2	
5	95% Alcohol	2	Rehydration
6	70% Alcohol	2	
7		2-3	Running tap water
8	Haematoxylene	5-10	Staining
9		2-3	Running tap water
10	Eosin	3-7	Counter stain
11	70% Alcohol	2-3 dips	
12	95% Alcohol	2-3 dips	
13	100% Alcohol	2	Dehydration
14	100% Alcohol	2	
15	Xylene	2	
16	Xylene	2	Clearing

According to Humason [22], the gonadal tissues were serially sectioned at a thickness of 5µ and leaving the sections to a water bath at a temperature of 40^0C. The section were placed on a glass slide and kept over-night on a slide drier hot plate at temperature of 20^0C. Then the sections were stained routinely with hematoxylene and eosin (Table 2). The stained sections were mounted on the glass slide with Canada balsam and covered a cover slip. The prepared section were examined under compound microscope (Zeiss Microimaging GmbH, PRIMOSTAR Trinocular Microscope) and photographic records were performed. Physical feature of the oocytes were observed under microscope and different developmental stages were identified.

RESULTS

Water quality parameters

Monthly some important environmental parameters were measured from different hapa and pond which were presented in Table 3. Water temperature was found to range from 21-35^0C during the study period among the treatments (P_o H_b and H_s). Water transparency measured only in the pond which was ranged

from 23.00 to 47.00 cm. The pH fluctuation was over the neutral level always but not exit above the optimum level for fish breeding and culture. DO and Alkalinity values ranged 2.25-5.60 mgL^{-1} and 73.00-127.00 mgL^{-1}. And also all inorganic nutrient conc. levels were suitable for breeding. Mean values of NH_3-N, NO_3-N, NO_2-N, PO_4-P and Chlorophyll-a found to range from 0.01-0.21 mg l^{-1}, 0.019-0.22 mg l^{-1}, 0.00-0.04 mg l^{-1} and 0.25-01.47 mg l^{-1} and 72.00-127.00 µg l^{-1}, respectively.

Table 3. Mean values (±SE) and range of water quality parameters in different treatments

Parameters	P_o	H_b	H_s	F- value	Level of sign.
Temperature (^{0}C)	29.25 ± 1.12 (21.00 – 35.00)	29.33 ± 0.99 (22.00 – 34.00)	29.19 ± 0.57 (21.00 -34.00)	0.007	NS
Transparency (cm)	36.58±2.10 (23.00-47.00)	-	-	-	-
pH	8.07 (7.12 – 9.00)	8.18 (7.15 – 9.25)	8.04 (7.09 – 9.30)	0.225	NS
Alkalinity (mg L^{-1})	101.33 ± 4.67 (73.00- 123.00)	99.33 ± 4.08 (72.00 - 120.00)	97.94 ± 2.75 (74.00 – 127.00)	0.206	NS
DO (mg L^{-1})	3.47 ± 0.28 (2.35 – 5.60)	3.76 ± 0.25 (2.43 – 5.47)	3.69 ± 0.15 (2.25 – 5.42)	0.031	NS
NH_3 (mg L^{-1})	0.101 ± 0.16 (0.02 – 0.21)	0.072 ± 0.13 (0.01 – 0.12)	0.109 ± 0.007 (0.01 – 0.18)	2.721	NS
NO_3 (mg L^{-1})	0.051 ± 0.017 (0.01 - 0.17)	0.044 ± 0.016 (0.01 – 0.18)	0.045 ± 0.009 (0.01 – 0.22)	0.071	NS
NO_2 (mg L^{-1})	0.005 ± 0.001 (0.00 – 0.02)	0.011 ± 0.003 (0.00 – 0.04)	0.009 ± 0.001 (0.00 – 0.03)	1.57	NS
PO_4 (mg L^{-1})	0.946 ± 0.088 (0.35 – 1.47)	0.929 ± 0.07 (0.25 – 1.11)	0.828 ± 0.041 (0.35 – 1.22)	1.27	NS
Chlorophyll-a (µg L^{-1})	90.91 ± 3.85 (76.00 – 114.00)	95.91 ± 4.05 (75.00 – 121.00)	93.61 ± 2.46 (72.00 – 127.00)	0.364	NS

*P_o- Pond, H_b- Big hapa, H_s- Small hapa

Plankton enumeration

Planktonic organisms were grouped as phytoplankton (x10^3 cells L^{-1}) and zooplankton (x10^3 cells L^{-1}). Mean abundance of both plankton in different successive months are shown in Fig. 2. Phytoplankton and zooplankton were varies 34.50-77.50 and 07.00-26.50 x10^3 cells L^{-1}. The pond water consist four groups with 38 genera of phytoplankton and also four groups with 12 genera of zooplankton.

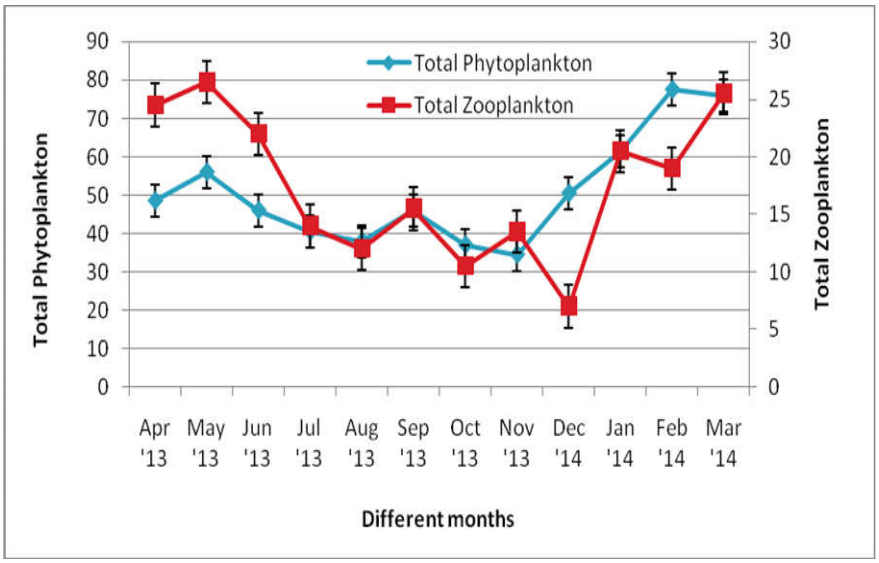

Fig 2. Month-wise mean (±SE) abundance of plankton numbers (x 10^3 cells L^{-1}) in different months in the pond water.

Month-wise obtained no. of fish from the hapa

Form the month-wise sampling in three small hapa (H_1, H_1 and H_3) and big hapa (H_b) shows an indication of breeding progression (Fig. 3). Among the all hapa fishes bred first time in H_1 and H_b. After then two times (months) bred in H_1 and H_3 and four times (months) in H_b. So along with the experiment fishes were bred two times in small hapa (both H_1 and H_3) and four times in big hapa (H_b). Therefore, breeding succession in small hapa, H_2 occurred once throught the experiment.

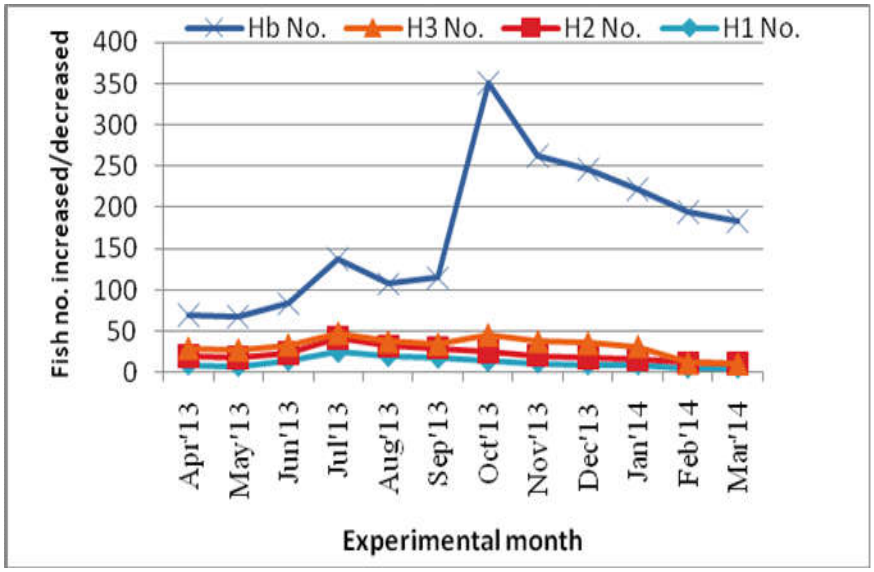

Fig 3. Month-wise obtained no. of fish increased/decreased trend in different treatment

Different sexual characteristics

Month-wise variation in the body weight (g), total length (cm), gonad weight (g), gonado-somatic index (GSI), fecundity and condition factor (K) of female and male were shown in Table 4 & 5. By the reason of sex separation, fishes were taken usually more than 1g and above. Because of <1g fish was more complex to collect and identify the gonad as well. In female, the highest body weight, total length, gonad weight, and GSI were 2.130±0.024, 6.140±0.085, 0.027±0.000, 1.305±0.037 in June but the highest condition factor was 1.111±0.015 in July. Hence, the fecundity varied between 1918±160.70 and 8071±428.70. In male, mean value of body weight, total length, gonad weight, GSI and K varied from 1.150±0.029 to 2.130±0.024, 4.900±0.092 to 6.140±0.085, 0.014±0.001 to 0.027±0.000, 1.054±0.062 to 1.338±0.036 and 0.927±0.035 to 1.085±0.127, respectively.

Table 4. In Female Darkina month-wise mean (±SE) body weight, total length, gonad weight, GSI (%) and Condition Factor (K).

Month	Body weight (g)	Total length (cm)	Gonad weight (g)	GSI (%)	Fecundity	Condition Factor (K)
April'13	1.910±0.111	5.410±0.124	0.230±0.013	12.480±0.575	2794±184.48	1.223±0.078
May'13	2.356±0.159	5.735±0.143	0.395±0.044	16.032±0.889	4582±204.64	1.223±0.021
June'13	3.256±0.179	6.493±0.103	0.684±0.071	20.079±1.180	8071±428.70	1.168±0.020
July'13	2.087±0.054	5.722±0.049	0.362±0.011	17.365±0.348	4318±188.63	1.111±0.015
August'13	2.706±0.147	6.050±0.109	0.400±0.049	14.216±1.098	4620±343.27	1.200±0.018
September'13	2.877±0.179	6.113±0.164	0.500±0.065	16.202±1.320	5940±419.32	1.245±0.059
October'13	2.667±0.153	5.850±0.104	0.358±0.037	13.189±0.833	4475±360.57	1.298±0.018
November'13	2.247±0.104	5.514±0.136	0.261±0.016	11.664±0.477	3288±252.26	1.347±0.040
December'13	1.932±0.053	5.41±0.062	0.182±0.005	9.468±0.234	2420±138.35	1.222±0.030
January'14	1.843±0.051	5.250±0.064	0.138±0.008	7.499±0.384	1918±160.70	1.280±0.039
February'14	1.928±0.074	5.440±0.082	0.171±0.007	8.964±0.365	2342±150.60	1.187±0.011
March'14	2.151±0.116	5.64±0.130	0.220±0.014	10.269±0.457	3223±263.47	1.191±0.036

Table 5. In Male Darkina month-wise mean (±SE) body weight, total length, gonad weight, GSI (%) and Condition Factor (K).

Month	Body weight (g)	Total length (cm)	Gonad weight (g)	GSI (%)	Condition Factor (K)
April'13	1.350±0.04	5.271±0.109	0.016±0.001	1.221±0.085	0.955±0.044
May'13	1.713±0.173	5.566±0.116	0.021±0.002	1.233±0.070	0.967±0.046
June'13	2.130±0.024	6.140±0.085	0.027±0.000	1.305±0.037	0.927±0.035
July'13	2.030±0.034	5.940±0.728	0.022±0.000	1.111±0.033	0.974±0.029
August'13	1.517±0.055	5.383±0.120	0.018±0.001	1.199±0.125	0.983±0.053
September'13	1.696±0.192	5.440±0.319	0.022±0.002	1.338±0.036	0.974±0.067
October'13	1.300±0.032	5.142±0.070	0.015±0.000	1.171±0.056	0.960±0.026
November'13	1.150±0.029	4.900±0.092	0.013±0.001	1.138±0.145	0.990±0.044
December'13	1.308±0.073	5.133±0.188	0.014±0.001	1.090±0.091	0.986±0.058
January'14	1.300±0.092	5.040±0.281	0.014±0.001	1.054±0.062	1.085±0.127
February'14	1.196±0.028	5.020±0.087	0.013±0.000	1.113±0.041	0.950±0.029
March'14	1.313±0.053	5.050±0.210	0.015±0.001	1.183±0.082	1.075±0.103

In fish, GSI & 'K' were be a sign of the gonad development attributes and physical condition of fish. These were changeable and depend on physical form and surrounding environment. The time-series changing pattern of mean GSI & 'K' values in between with male and female were revealed in Fig 4 & 5.

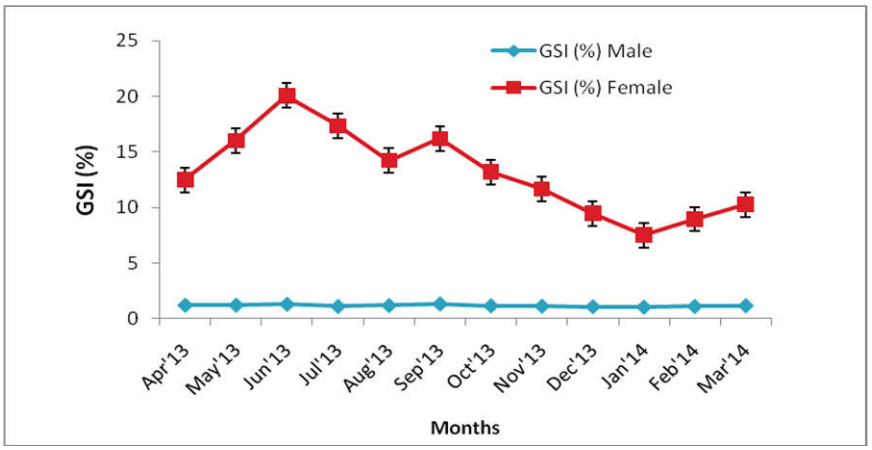

Fig 4. Monthly variation (±SE) of GSI (%) of male and female Darkina, *Esomus danricus*

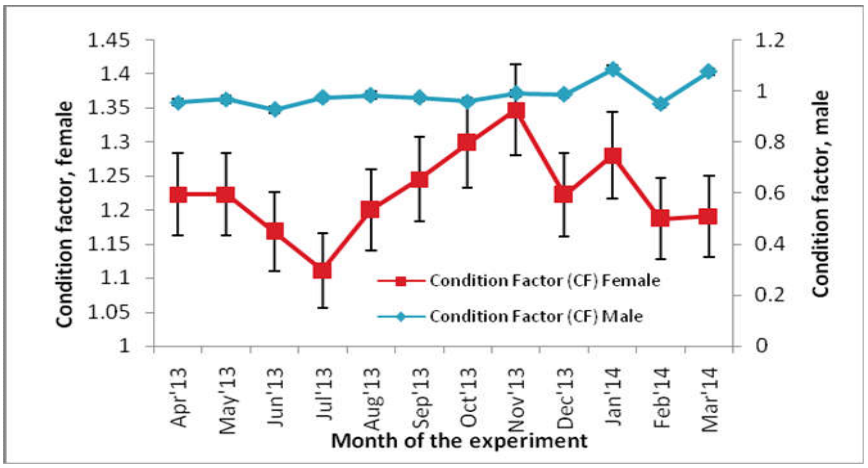

Fig 5. Monthly variation (±SE) of Condition Factor (CF) of male and female Darkina, *Esomus Danricus*

Occurrence of different ova size

By the help of ocular and stage micrometer categorized four sizes of ova. The dimension of ova was assembled: immature ova (0.0-0.15 mm), maturing ova (0.16-0.30 mm), mature ova (0.31-0.45 mm) and ripe ova (0.41-0.60 mm). These oocytes were unevenly distributed in months (Fig. 6).

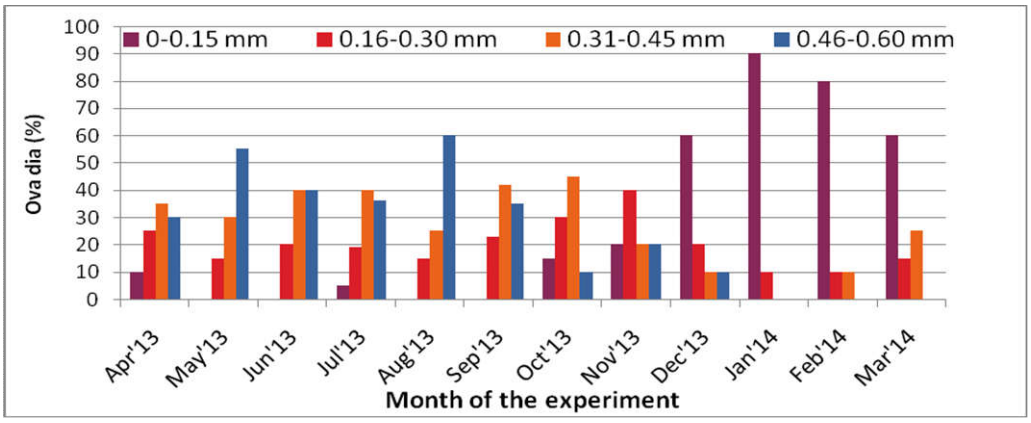

Fig 6. Monthly percentage (%) of different ova dia frequency of Darkina, *Esomus danricus*

The scattered diagram obtained with plotting between fecundity and gonad weight showed positive relationship. The relation with them was established to be polynomial of second order of gonad weight in a formula as: $Y = -4817.x^2 + 11044.x + 927.4$ (Fig. 7).

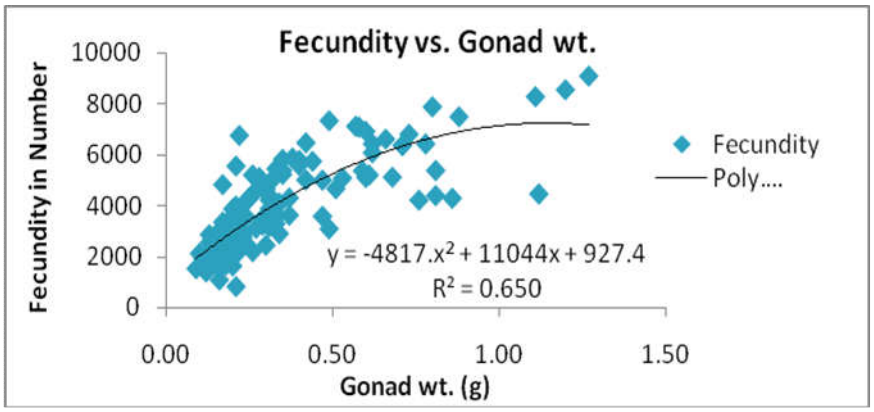

Fig 7. Relationship between fecundity and gonad weight of Darkina, *Esomus danricus*

Different stages of gonad maturation

Table 6. Macroscopic & histological description of gonad maturation stage of female darkina

Maturity stage	Macroscopic description	Histological description
Immature	Ovaries very small not to visible by naked eye, thin, thread like pale in color, occupying a small part of the body cavity.	The ovary was consists of oogonia cells and primary oocytes. The wall of the ovary thick (Fig. 8).
Maturing	Larger than previous stage, oocytes become visible. Eggs are oval in shape and sometimes slight unequal of two lobes.	Primary oocytes lager and more in number. The wall of ovary was thicker and some oocytes going to vitellogenesis (PVO) with yolk granules on the cytoplasam (Fig. 9).
Mature	Paired ovaries free, whitish and clearly visible. These are distended occupied about 2/3 of the abdominal cavity.	Nucleus could not be observed due to the granular structures filled up the entire cytoplasm and more yolk accumulation (SVO). The membrane of the nucleous dissolved (Fig. 10).
Ripe	Ovaries smooth and less firm, yellowish in color and larger in size. Occupying almost entire body cavity and eggs start to become outside with the slight pressure on abdomen.	Numerous hydrated oocytes and more abundance Post vitellogenic oocyte (PsVO) and few primary vitellogenic oocyte (PVO) & secondary vitellogenic oocyte (SVO) present (Fig. 11).
Spent & resting	Ovary smaller than previous. Shrunken in size. Flaccid.	Post vitellogenic oocyte (PsVO) absent. Oogonia scattered and few primary oocyte present. Large empty space also seen (Fig. 12).

Table 7. Macroscopic & histological description of gonad maturation stage of male darkina

Maturity stage	Macroscopic description	Histological description
Immature	Testes are very thin, paired, thread-like, transparent, emerge single line.	A lot of spermatogonia (S) visible. Contain the occasional spermatocyst (Fig. 13).
Maturing	Testes darker and enlarged than before, whitish in color. Testes not connected but look joint.	Spermatocytes(SC) more in number with the spermatogonia (S), sperm ducts more visible than in immature stage (Fig. 14).
Mature	Two lobes of testes were translucent and convoluted like twisted rope	Spermatocytes(SC) more with spermatids. The sperm duct which is clearly seen in the low magnification micrograph (Fig. 15).
Ripe	Testes milky in color and less translucent. With pressure to the abdomen, milt flows out.	Few spermatozoa are present in sperm duct which are distended (Fig. 16).
Spent & resting	Testes became flabby, shrunken in size and weight, facade in darker.	Sperm ducts empty with few residual spermatozoa. Connective tissues beginning to form (Fig. 17).

Histological slides

Darkina (female)

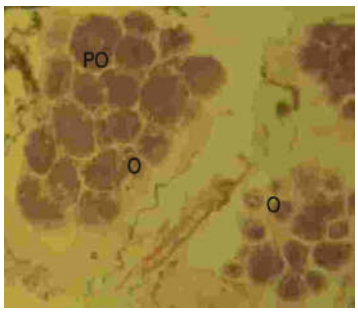

Fig 8. Immature stage

Fig 9. Maturing stage

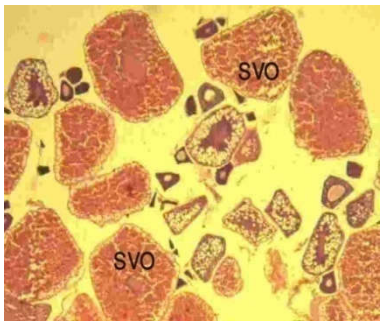

Fig 10. Mature stage

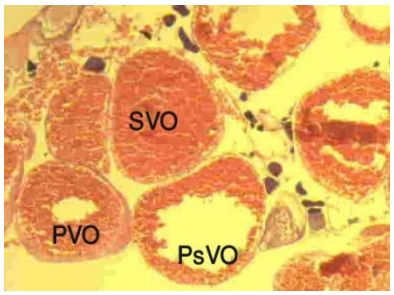

Fig 11. Ripe stage

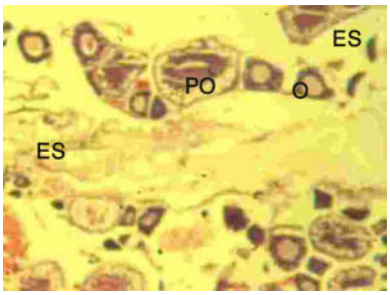

Fig 12. Spent & resting

O- Oogonia, PO- Primary oocyte, PVO- Primary vitellogenic oocyte, SVO- Secondary vitellogenic oocyte, PsVO- Post vitellogenic oocyte, ES- Empty spaces

DARKINA (male)

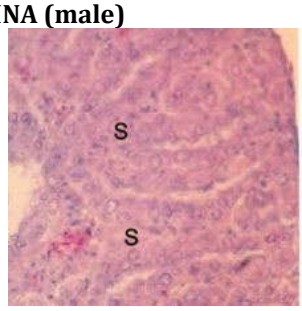

Fig 13. Immature stage

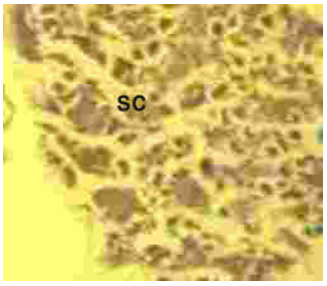

Fig 14. Maturing stage

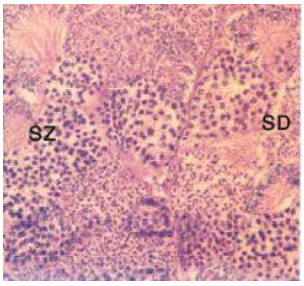

Fig 15. Mature stage

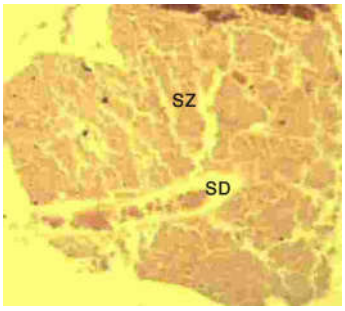

Fig 16. Ripe stage

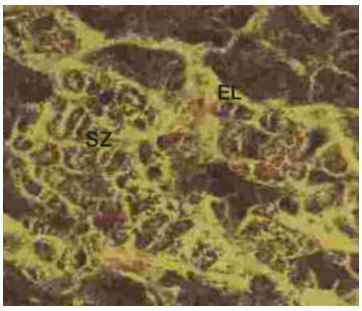

Fig 17. Spent & resting

S-Spermatogonia, SC-Spermatocytes, SD-Sperm duct, ST-Spermatids, SZ- Spermatozoa and EL- Empty lumen

DISCUSSION

The biological information of darkina, *Esomus danricus* is inadequate. To get an effective knowledge of darkina biology were taken initiative comparatively a close observation in a big pond within total thirty seven hapas. The pond consists of polyculture previously. Perhaps no work has been done on reproductive biology of darkina in hapa. No feed was applied for darkina. They are dependent on natural food from the pond waters. So, it seems that fertilizers should be applied regularly in the pond. From this point of view, fertilizers were applied regularly early two months. But the recommended dose of fertilizers (urea & TSP) generates algal blooms which obstructs water passing through the meshes of hapa. So, application of fertilizer in the pond was irregular basis other rest of the ten months. Considering the annoyance of fishes, hapas were not cleaned except sampling time. Because it was a motto to know the breeding performance of that fish. Subsequently, in the sampling time most of the hapa was cleaned by soft brushes for their friendly atmosphere.

Water quality in all the hapas and pond were mainly affected by seasonal condition in the similar way. While variations in mean values of all parameters (except transparency) in hapas were recorded similar. Transparency was observed only in the pond water. Though transparency in fish culture ponds is affected by a number of factors like silt, clay particle, water depth, rainfall, organic matter and planktonic organisms [16]. In the experiment, transparency has been changed particularly by the presence of phytoplankton population. The transparency values in the pond were also plausibly ascribed by the chlorophyll-a concentrations. All the physical and chemical parameters of waters were found both hapa and ponds within the suitable ranges for breeding and there was no unexpected change in any parameter. Lagler [28] believed that spawning results from a combination of changes especially those involving temperature, pH, oxygen and dissolved chemicals. No significant difference ($P<0.05$) of water quality parameters among the different treatments. The values of water quality parameters were revealed to be within the acceptable ranges for fish culture and as well breeding [10, 17, 26].

Darkina were bred four months of the year. Among the small hapas H_1, H_2 and H_3 were attained breeding in June-July, July & October, respectively. But in the large hapa were sampled every month and recording total weight and no. of the fish before release. From that observation, fishes were bred June, July and October in the big hapa (H_b). Last two months could not perform sampling from H_3 hapa because those hapa torn up and entered another tilapia fishes from the pond. The fishes of darkina were not performed breeding all fishes at a time in the same population. It may be called multiple spawner. After breeding, all the spawned fish seems not to die. Habitually after breeding, the spawned fishes are feeble. From this behavior it is not to easy comment, the fate of fishes after breeding. It may be come out the clandestine breeding behavior from aquarium (with camera) based culture system.

Plankton can show remarkable changes in population density from day to day due to their short life cycle and capacity to produce quick changes in pond water [53]. Perpahs phytoplankton is the key factor which manipulating the quality of water in pond. In the experiment, phytoplankton was dominated with Chlorophyceae followed by Cyanophyceae, Bacillariophyceae & Euglenophyceae and zooplankton were dominated with rotifera. This observation is similar to the abundance of plankton with the findings of Kohinoor [25] & Kunda [27]. Euglenophyceae and copepoda are poorly observed. The phytoplankton & zooplankton succession pattern were similar in the eight months but other four months October, November, December and February were showed inversely. Increasing primary productivity following fertilizer usually results in greater zooplankton abundance [10, 51] . In pond culture, Biological control is the friendliest method for regulating phytoplankton abundance [49].

In female, GSI recorded highest in June and afterward July. And also the body weight, total length, and gonad weight were high in June and the lowest values observed that all characteristics were low in January including GSI. Generally fishes were bred which month reaches peak GSI values. GSI value co-related with breeding as well. And also observed the GSI of female was higher than male. That finding was co-related with Kohinoor [25] and Gupta & Banerjee [21].

In male, gonado-somatic index (GSI) documented two peaks in June and September during the experimental period. The lowest was in January and then December. The mean values of GSI were increased and decreased pattern usually followed by the female. And also the body weight, total length, and gonad weight were high in June. Initially increased GSI and then after bred gradually decreased trend followed and again increased in September. The both sexes of fishes were more or less similar with another small fish, *Amblypharyngodon mola.* That month was second breeding period and after then fluctuate the mean values of GSI to the end.

Change in condition factor with increasing weight and length in this research showed that average weight of fish does not increase in direct proportion to the cube of its length [43]. A decrease in the condition factor, is considered a mirror image of depletion in energy reserves because these indices are positively associated to muscle and liver energy content [32]. Young of the year tend to distribute a large amount of energy in growth, while adults put a substantial part of their energy in reproduction process or increasing weight or survival in environmental stress. It was observed that smaller size individual in first collection present high growth rate and inversely linked to condition factor.

In this experiment, male shows low (0.927) condition factor in June and high (1.085) in January and also female shows low (1.111) in July and high (1.347) in November. Das & Dewan [14] recorded the values of condition factor of darkina range from 0.87-1.31 which is the similarity of present study. This result was in agreement with the findings of Mustac and Sinovcic [34] that expressed in sardine (*Sardina pilchardus*) fish from the inactive phase of reproductive cycle was generally in better condition (K=0.8234) than the fish caught during spawning season (K=0.7409). On the other hand, the condition factor (K) is a parameter of the state of well-being of the fish based on the hypothesis that heavier fish of a particular length are in a better physiological condition [5].

The fecundity of a fish is defined as the number of eggs that are likely to be laid during a spawning season [6]. The reproductive potential *i.e*. fecundity is a key characteristic that plays a significant role in evaluating the commercial potentials of fish stocks [19]. Successful fisheries management including practical aquaculture relies on having an accurate assessment of fecundity to understand the recovery ability of fish populations [28, 37, 50]. The fecundity and its relation to female size make it possible to estimate the potential egg output [11] and the potential number of offspring in a season and reproductive capacity of fish stocks [41]. It was observed in some cases that the fecundity of some larger fishes was much less than that of some smaller fish. This type of variation was also recorded by different researcher Doha & Hye [18], Karim & Hossain [23], and Ahmed *et al* [1] .

The fecundity of darkina is followed by the GSI & gonad weight as well. In small fish darkina carried enormous amount of eggs which ranged from 1918±160.70 to 8071±428.70. During the experiment, fecundity observed large amount of eggs in those breeding months. The fecundity of mola observed 1,291-12,737, 1,014- 9,690 & 3,785-12,590 by Saha *et al*. [46], Gupta and Banerjee [21] and Mondal & Kaviraj [31] respectively. Those findings more or less followed with small fish, darkina in the culture pond.

The progressive change observed in the intra-ovarian diameter for a period not less than a year can give an idea of the spawning periodicity of the fish studies [9]. Usually ova dia changed according to maturation phase. In the present study, immature ova were found the highest level (90%) in January and decreased onward. The immature (0-0.15mm) ova were distinguished amount during in December-March. The maturing ova were distributed throughout the experiment period. Whereas, the mature ova of also distributed throughout the experiment period except January. Ripe ova were recorded first nine months of the experiment but peak in May & August. Before the breeding season were observed peak level of ripe ova and wholly absent in January-March.

The scatter diagram obtained from the fecundity and gonad weight showed a linear relationship. In the determination of this relation, fecundity was taken as dependent variant, while gonad weight as independent variable. The co-efficient of determination (0.650) was found to be significant at 1% level and more than 65% of total variation in fecundity may be explained by using such an equation $Y= -4817.x^2 + 11044.x + 927.4$. That linear relationship between fecundity and ovary weight were examined in favor of mola and puti concur with the findings of Kohinoor [25] & Mustafa *et al*., [36].

Five gonad maturation stages were established with description through microscopic and histological examination of female which has been presented in Table 6. In female immature gonad were found most of the month of the year. In winter season (December- January), immature gonad was more prominent which are very small not to visible by naked eye and lots of oogonia were visible. Oocytes started maturing from March, which become enlarge & visible and abundance primary oocytes & some primary vitellogenetic oocyte (PVO) with yolk granules. As the rainy season approaches in May, mature oocytes whitish & clearly visible and present more secondary vitellogenetic oocyte (SVO) which catches up to the end of the rainy season in September. Ripe and running oocytes yellowish & larger in size and observable more post vitellogenetic oocyte (PsVO) with few PVO & SVO come across during the peak of the rainy season in July. And also absent from January, gesture the end of the spawning. Spent and resting gonads looks flabby and shrunken in size and present few residual oogonia & primary oocyte at the end of the autumn season from November.

Gonad maturation stages were recognized with narrative through macroscopic and histological inspection of male which has been presented in Table 7. Similar trend was observed in male. This study is supported by the findings of Shinkafi *et al.* [48]. Immature gonad was very tiny & thread like and more spermatogonia were visible. Maturing gonad looks darker & enlarge and abundance spermatocytes with spermatogonia. Mature gonad was translucent and consists of plenty spermatocytes with spermatids. Ripe and running testes were mostly milky color and visible few spermatozoa. Spent and resting gonads looks flabby and shrunken in size and present few residual spermatozoa.

In the present study, gonad increases from immature to the maturing and was the largest in mature stage. They declined from ripe and running to spent and finally up to the resting stages and back to immature when the cycle commences again. Gonad maturation stages of the two sexes recorded, spawning of *E. danricus* has its peak from June to October. Gonad development and morphological changes in color, shape and size occurred during the maturation process and follow the similar pattern in most oviparous fishes [7].

Taking into account on microscopic and histological examination of *E. danricus*, five stages of gonad maturation, with an annual spawning season which occurs two peaks in June-July & September-October. It was also found to be a multiple spawner, releasing its reproductive products in batches over a long spawning period of about three months, which is June–October. Consequently, there is plenty opening for diverse more close natal works and in addition to set up camera with big aquarium on behalf of that fish.

ACKNOWLEDGEMENTS

All expenses of the research work were covered by the WorldFish through its "Cereal Systems Initiative for South Asia (CSISA-BD)" project. This work could not have been done without hard work of Lab Technician and Field Staffs of Fisheries Field Laboratory, BAU, Mymensingh who's carried out the experiment. The Director and Senior Nutrition Adviser of WorldFish are acknowledged for his cooperation during the study.

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