Induced Breeding of *Barbodes Gonionotus* (Bleeker, 1850), Pond of Hinthada Department of Fishery, Hinthada Township, Ayeyawady Region

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Abstract

The study was conducted to know the induced breeding technique of silver barb (*Barbodes gonionotus*) are chosen for the experiment at department of fisheries in Hinthada. The present study, silver barb in mature females and males by administering hormone of single dose in female and male by intra-peritoneal injection of mixture Cinnafact and Motilium at a dosage of 4.625 kg and 4.22 kg for silver barb of body weight. There were seven main periods of embryogenesis such as zygote, morula, blastula, gastrula, segmentation, pharyngula and hatching period. Six larval development stages were recorded in silver barb hatching period as twelve hours larvae, one day larvae, two days larvae, three days larvae, eight days larvae and twenty-one days larvae. Hatching occurred in silver barb at range of about 13 to 14 hours after egg fertilization. Fish specimens were used to assess the length-weight relation and condition factor of them during the study period. The exponential value 'b' was found to be *Barbodes gonionotus* **(**3.8418) that expressed as the positive allometric growth (b>3). The value of coefficient of determination (R^2=0.793).The value of R^2 was close to 'one'.

Keywords: induced breeding, silver barb, development stages,

INTRODUCTION

Fishes are cold - blooded animals typically with backbones, gills and fins (FAO, 2003). In Asian countries, fish is considered second to rice in the diet of Myanmar people. Myanmar has impressive freshwater capture fisheries and aquaculture as well (FAO, 2010). Lakes, ponds, reservoirs and river system which can provide freshwater fish population (FAO, 2003).

Fish are valuable sources of high-grade protein and other organic products. They occupy a significant position in the socio-economic fabric of the South Asian countries by providing the population not only the nutritious food but also income and employment opportunities (Talwar and Jhingran, 1991).

Induced breeding is the highly reliable techniques to reestablish the declining natural stock as well as to meet the raising demand of seeds to the farmer (Anonymous, 2014).

Induced breeding is a technique by which the economically important fish (which generally as not breed in captive condition) are not bred through artificial stimulation (Paul and Chanda, 2014). In Myanmar induced breeding of indigenous carps during 1967 by pituitary of fish seed is the first successful achievement (Chaudhuri, 1960). Induced spawning refers to a process in which some stimulants, hormones or pituitary extracts are injected in the brood fishes, which do not spawn in the closed water bodies, causing the fishes to spawn (Bhuiyan *et al.,* 2006).

B. gonionotus is native to Southeast Asia and can be found throughout the Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Java (Kottelat, 1998). *Barbodes gonionotus* is one of the five most important aquacultured freshwater species in Thailand. This

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species feeds mostly as an herbivore on aquatic plants but will also consume invertebrates. *B. gonionotus* is known to migrate upstream during the rainy season in its native range, and can often be found in flooded forests during high water events (Rainboth, 1996).

Van Der *et al*., (1992) stated that the reproduction of fish is controlled by the coordinated actions of various hormones along the brain hypothalamus pituitary gonad axis with the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) having central roles.

Cinnafact is the most common form of LHRH (Luteinizing hormone, Releasing hormone), currently available is the chemical known as 'buserelin acetate'. This is sold as a drug for human usage under the trade name 'Cinnafact'. Cinnafact has been used successfully in the spawning of a wide range of fish species in many countries. When using Cinnafact to induced fish spawning it is necessary to mix it with domperidone maleate which enhances the effect of the Cinnafact and improves spawning success. It is sold as a drug for human use under the trade names of 'Motilium' and Mirax (Meenakarn and Funge-Smith, 1998).

The determination for grouping of fish, where the analyzed samples were classified into three groups i.e., light, isometric and heavy (Smith, 1996). The light group was determined when the value of $b < 3.0$. In this group, the length growth was not proportionate to the increase in weight when the weight gain is more than an increase in length, the fish falls in heavy group with $b > 3.0$. The ideal value of $b=3.0$ indicates the fish were having the isometric growth of equal increment of both parameters (Salam *et al.,* 2005).

Condition factor (K) is widely used in fisheries and fish biology studies. 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 (Froese, 2006).

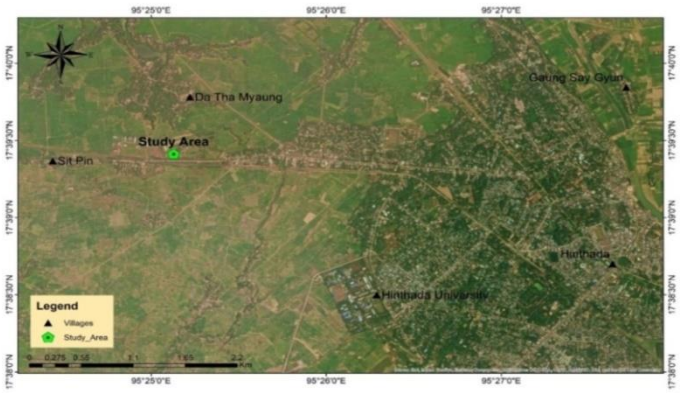
The paper is conducted with the following objectives:

- to study the induced breeding of *Barbodes gonionotus*
- to observe the embryonic and larval development of *Barbodes gonionotus*
- to determine the growth rate and length of *Barbodes gonionotus*

MATERIALS AND METHODS

Study area and study site

The present study was conducted in Hinthada Department of Fishery, Hinthada Township, Ayeyawady Region. It is located between latitude 17˚ 39′ 22″ N and longitude 95˚ 25′ 16″ E.



(Source: Geology Department, Hinthada University, 2022)

Fig.1. Map of the study area

Materials using for induced Breeding

Medicinal hormone such as Cinnafact and Motilium were used for injection. Some materials such as distilled water, syringe, dissecting microscopes, digital balances and drag nets were also used during the artificial induced breeding.

Ponds and tanks for culture

Brood stock pond, spawning pond, hatching jar or tank, larval tank and nursery ponds were used to culture in the study area. Some concrete tanks such as breeding tank and rearing tanks were used during the induced breeding of fish in the study site.

Selection of breeders

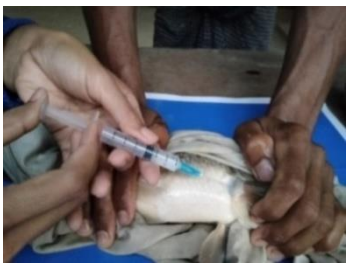
The breeders of *Barbodes gonionotus* were collected to breed for spawning. These species were taken by drag net from brood stock pond. Males and females spawners were selected about one year old for *B.gonionotus*. A total of fourteen males and seven females of *B.gonionotus* were chosen as spawners for one batch.

Using of medicinal hormone and injection for spawning

The breeder females were given injection of Cinnafact 15µ per kg, Motilium 5mg per kg and distilled water 1 CC per kg of fish body weight. Half a dosage of hormones was also prepared for male breeders.

Injections were usually made intra-peritoneal at the base of the pectoral fin of breeders. The injecting needle was inserted into the muscle of fish about 1cm in depth at the angle of 45˚ to the body longitudinal axis (Plate 1).

A.Injecting in male breeder



B.Injecting in female breeder

Plate 1. Breeding synthetic hormone injected of recorded fishes

Preparations and procedure of injection

Injection was carried out at the morning for silver barb in the study area. After injection, breeder fishes were kept into concrete tank separately by spraying water to stimulate for mating at ambient temperature 24˚C.

In silver barb, the eggs were transferred to the hatching jar and the fertilized eggs were found 14 hours later after the laid eggs. The fries were transferred from hatching jar to the concrete tank after two days of hatch. After three days, the fries were transferred to the rearing pond (Plate 2).


A. Eggs introduced into hatching jar


B. Larva concrete tank


C. Larva introduced into rearing pond

Plate 2. Incubation tank of eggs and larval rearing pond

Estimating of fertilization, hatching and survival rates

In petri dish, unfertilized eggs were removed and the numbers were also counted to estimate the fertilization rate from the samples. When hatching was completed, the death eggs were removed and counted by visual observation and recorded to estimate the hatching rate. Then, survival rate of the number of live fries were observed. These rates were determined by using the following formulas - (Kaur and Dhawan, 1997).

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs in sample}}{\text{Total number of eggs in sample}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings in sample}}{\text{Total number of fertilized eggs in sample}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of survived fry in sample}}{\text{Total number of hatchlings in sample}} \times 100$$

Observation of embryonic and larval stages

Fertilized eggs were collected with the help of a feather. Descriptions of the developing stages were made by examining live specimens under dissecting microscopes. Photographs of the developmental stages of eggs and larval were also taken (Plate 3. A).

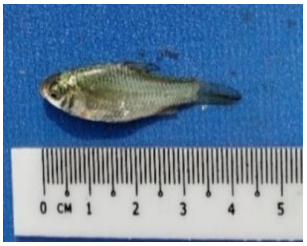
Samples of eggs were taken prior to hatch at every 15 minutes intervals were taken for further studies. Developmental time from fertilization was round to the nearest minute until the morula stage and then to the nearest hour. The age of the larvae was denoted an hour after activation.

Measurement of length and body weight

The total length and body weight of fish were recorded in fresh condition. Total length of each fish species was measured from the tip of the snout (mouth closed) to the extended tip of the caudal fin using a plastic ruler. Body weight was weighted to the nearest gram with a digital balance after removing the adhered water and other particles from the surface of the body (Plate 3. B and C).

A. Under microscopes examing eggs

B. Weighting of fries



C. Measurement of fish

Plate 3. Observation of under microscope in eggs and measurement of length and body weight

Calculation of length -weight relationship

The following method was adopted for the assessment of various measurements of parameters and their relationships length-weight relationship: (King, 1996).

The length- weight relationship was calculated using the least squares regression on log- transformation of the equation, W=a*L b (FAO, 1992) and all weights (g) and total lengths (cm) were fitted to these equations.

Where, L=body length of the specimen

W=body weight of the specimen

a and 'b' are the intercept and slope (-exponent) of the length-weight curve, respectively (King, 1996).

After logarithmic transformation of the relation (log W=log a+ b log L). Where parameter 'a' and 'b' were determined via least-square linear regression where 'b' is an exponent with the value nearly always between 2 and 4 and often close to 3.

The value b=3 indicates that the target species grows symmetrically or isometrically without changing body proportions. Values other than 3 indicate allometric growth: if b>3, the growth is considered as positive allometric and if b< 3 is negative allometric.

Condition factor (K)

The value of condition factor (K) was calculated with the following equation cited by Williams (2000).

$K=W/L^3\times100$

W=weight of the fish in grams

L=standard length of the fish in centimeters

The value of K close to one is considered as good in assessing the well-being state.

Data Analysis

Total body length (TL) and body weight (BW) of *Barbodes gonionotus* were recorded to the nearest centimeter and gram respectively. All data were statistically analyzed by using computer Microsoft Excel programme (2010).

RESULTS

Selected breeders

Selections of breeding sets were constructed as two males and one female. The total weight of 4.22 kg and 4.625 kg for *B.gonionotus* during the study period.

Deposition of eggs and production of fries in silver barb

In silver barb, the ovulation time lasted from 6 to 8 hours in ambient temperature 24˚C. In one 5CC injecting tube were received the number of eggs 620, in fifteen 5CC injecting tubes were received 9300 eggs, in fifteen steel cups were received 139500 eggs, thirty-one purified drinking water were received 4324500 eggs. The fertilized eggs were adhesive, sticky, demersal and slight brownish in color but unfertilized eggs were spherical in shape and brownish in color. In petri dish, 100 eggs were observed 85 fertilized eggs and in 71 fries were survived 53 fries. The eggs were counted and were placed in each hatching jar. A continuous flow of water was maintained. The hatchlings came out after 24 -29 hours. The hatchlings were kept in each hatching jar for three days. After three days the hatchlings were taken rearing pond.

Fertilization, hatching and survival rates

The performance of *Barbodes gonionotus* breeders subjected to induce spawning during this investigation. In silver barb, the average fertilization rate of the eggs, hatching rate of fertilized eggs and survival rates of fries were obtained 85%, 84% and 74.65% at the ambient temperature 24˚C.

Table1. Spawning and hatching performances of silver barb

No.of eggs	No.of fertilized eggs	No.of hatchlings	No.of survival fries	Fertilization rate	Hatching rate	Survival rate
4,324,500	3,675,825	3,070,395	2,291,985	85%	84%	74.65%

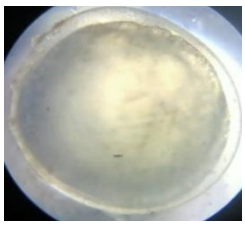
Developmental stages of silver barb

In general, the development of silver barb can be divided into two stages: the first is embryonic stage and the second is the post hatching stage (larval stage)**.**

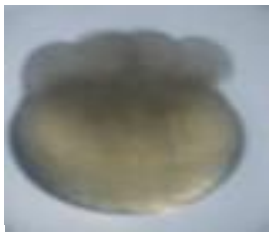
Embryonic stages

The embryonic stages were divided into seven main periods: zygote (cleavage), morula, blastula, gastrula, segmentation, pharyngula and hatching period (Plate 4).

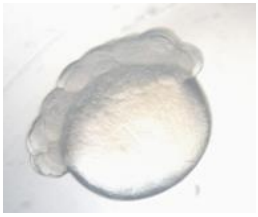
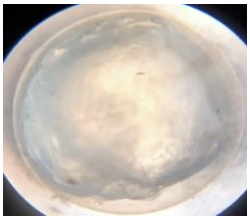
A. One cell stage



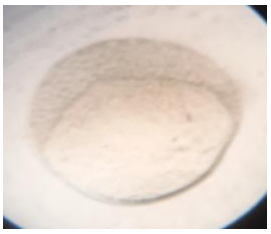
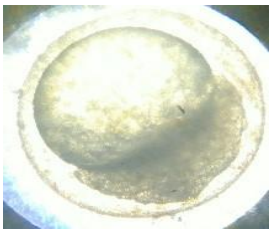
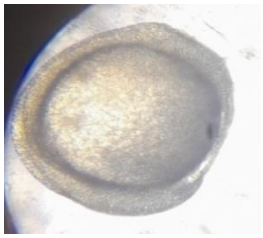
B. Two cell stage



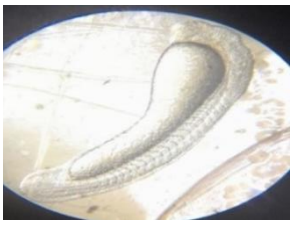
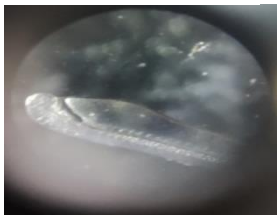
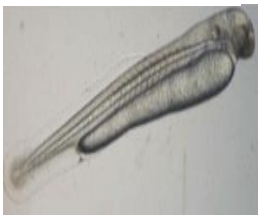
C. Four cell stage

(D) Eight cell stage (E)Sixteen cell stage F.Thirty-two cell stage

G.Morula H. Blastula I. Gastrula

J. Segmentation K. Pharyngula stage L.Hatching period

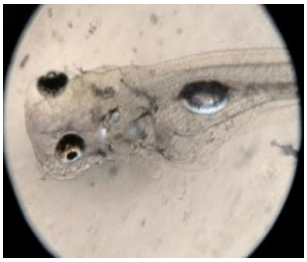
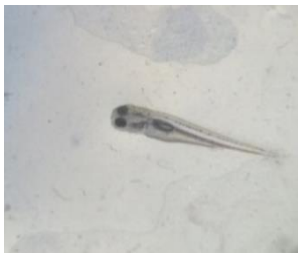
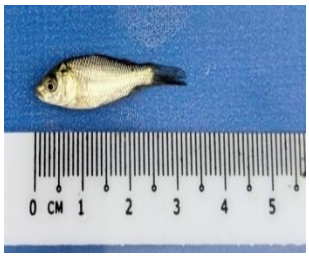
Plate 4. Embryonic development of *Barbodes gonionotus* (4X)

Larval stages

Different developmental stages were distinguished in the silver barb larvae, starting from the twelve hours larval stage to the twenty-one days larval stage (Plate 5).



A.12 hours old larva B. One day larva C. Two days larva

D. Three days larva E. Eight days larva F. Twenty one days larva

Plate 5. Larval Development of *Barbodes gonionotus* (4X)

Relationship between body weight and total length

The thirty individual of *Barbodes gonionotus* were observed with the measurement and body weight to calculate the relationship between body weight and total length. The regression analysis of length-weight relationship for silver barb to the length - weight parameters 'a', 'b' and the coefficient of determination 'R^2'. The parabolic equation of body weight (BW) and total length (TL) relationship of *Barbodes gonionotus* obtained the value b= 3.8418, and (> 3), the growth was also considered as positive allometric.The value of condition factor 'K' was found to be 1.05. R^2 and 'r' values of total length and body weight were 0.793 and 0.890 respectively (Table 2 and Fig.2.).

Table 2. Weekly total length and weight relationship (LWR) and condition factor (K) of silver barb

Sr.No	Length(X)	Weight(Y)	Log of length	Log of weight	XY	X^2	Y^2	al^b	Kvalue
1	3.5	0.4	0.5440	-0.3979	-0.2165	0.2960	0.1583	0.3701	1.0807
2	3.2	0.2	0.5051	-0.6989	-0.3530	0.2551	0.4885	0.2623	0.7624
3	3.7	0.8	0.5682	-0.0969	-0.0550	0.3228	0.0093	0.4581	1.7459
4	3	0.1	0.4771	-1	-0.4771	0.2276	1	0.2047	0.4884
5	3	0.1	0.4771	-1	-0.4771	0.2276	1	0.2047	0.4884
6	5.7	1.8	0.7558	0.2552	0.1929	0.5713	0.0651	2.4102	0.7468
7	4.5	0.9	0.6532	-0.0457	-0.0298	0.4266	0.0020	0.9719	0.9259
8	3.5	0.4	0.5440	-0.3979	-0.2165	0.2960	0.1583	0.3701	1.0807
9	3.5	0.4	0.5440	-0.3979	-0.2165	0.2960	0.1583	0.3701	1.0807
10	3.5	0.6	0.5440	-0.2218	-0.1207	0.2960	0.0492	0.3701	1.6211
11	5	1.9	0.6989	0.2787	0.1948	0.4885	0.0777	1.4569	1.3041
12	4.5	1.7	0.6532	0.2304	0.1505	0.4266	0.0531	0.9719	1.7490
13	4	0.9	0.6020	-0.0457	-0.0275	0.3624	0.0020	0.6181	1.4558
14	5	0.7	0.6989	-0.1549	-0.1082	0.4885	0.0239	1.4569	0.4804
15	5	1.3	0.6989	0.1139	0.0796	0.4885	0.0129	1.4569	0.8922
16	7	3.4	0.8450	0.5314	0.4491	0.7141	0.2824	5.3067	0.6406

Table 1. Continued

Sr.No	Length(X)	Weight(Y)	Log of length	Log of weight	XY	X^2	Y^2	al^b	Kvalue
17	5	1.3	0.6989	0.1139	0.0796	0.4885	0.0129	1.4569	0.8922
18	5	1.4	0.6989	0.1461	0.1021	0.4885	0.0213	1.4569	0.9609
19	3.5	0.4	0.5440	-0.3979	-0.2165	0.2960	0.1583	0.3701	1.0807
20	3.2	0.2	0.5051	-0.6989	-0.3530	0.2960	0.1583	0.2623	0.7624
21	5	2	0.6989	0.3010	0.2104	0.4885	0.0906	1.4569	1.3727
22	5	1.8	0.6989	0.2552	0.1784	0.4885	0.0651	1.4569	1.2354
23	5.3	2.1	0.7242	0.3222	0.2333	0.5245	0.1038	1.8224	1.1522
24	4	0.9	0.6020	-0.0457	-0.0275	0.3624	0.0020	0.6181	1.4558
25	3.5	0.3	0.5440	-0.5228	-0.2844	0.2960	0.2734	0.3701	0.8105
26	5.5	1.9	0.7403	0.2787	0.2063	0.5481	0.0777	2.1011	0.9042
27	4.2	0.7	0.6232	-0.1549	-0.0965	0.3884	0.0239	0.7456	0.9387
28	4.5	1.3	0.6532	0.1139	0.0744	0.4266	0.0129	0.9719	1.3375
29	4.5	1.2	0.6532	0.0791	0.0517	0.4266	0.0062	0.9719	1.2346
30	5	1.1	0.6989	0.0413	0.0289	0.4885	0.0017	1.4569	0.7550
	131.1	32.4	18.9336	-2.9156	-0.9073	12.1922	4.5506		1.0585

b	nExy	minus	EXEY	-27.21981602	-55.20341057	27.98359455	
	nEx2	minus	Ex)^2	365.7675095	358.4835317	7.283977745	3.841801215 b
A	Ey	minus	bEX	-2.915622583	72.73934014	-75.65496272	
	n					-2.521832091	0.003007 a

Antilog of obtained a value

K value W/W^

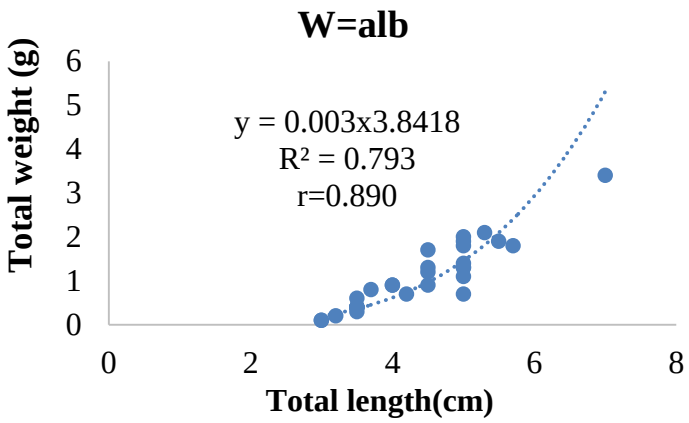


Fig 2. Parabolic form of *Barbodes gonionotus*

DISCUSSION

The present study investigated the artificial hormone induced fertilization of silver barb, *Barbodes gonionotus* in Hinthada Fishery Department. They were the commercial productions of fish species for human consumption in Asia including Myanmar.

A total of fourteen males and seven females of *Barbodes gonionotus* were selected for artificial breeding by using the hormone stimulates injection. The fertilization, hatching and survival rates were affected by the different temperature.

Basak *et al*., (2014) described that induced breeding of silver barb with pituitary gland at 6mg/kg female and 3 mg/kg male was injected. Sex ratio of 1:5:1 (male: female) for induced breeding. The ovulation time was 5 - 6 hours after injection. The hatching period was observed to be 13:40 -14:00 hours at water temperature 27.5 - 30.5˚C. But Udit *et al*., (2014) stated that the induced breeding of *Puntinus sarana* with ovatide at 0.2 ml per male (180g) and 0.3 ml per female (232-240g) was achieved. Sex ratio of 2:1 and 1:1 (male: female) were maintained in two trails. The interval between injection and spawning was 8-9 hours. The hatching period was observed to be 15-16 hours at water temperature 26.5-28.5˚C. The present study of induced breeding of silver barb, injection was given by the using of Cinnafact mixed with Motilium Tablet for production of silver barb and sex ratio of 2:1 (male:female) for induced breeding and hatching was about 13:35 to 14:00 hours in ambient temperature 23˚C. These might be due to temperature difference.

In the present findings, indicated that both the fertilization, hatching and survival rates of silver barb were 85%, 84% and 74.65% whereas Udit *et al.,* (2014) stated that the average fertilization, hatching and survival rates of *Puntinus sarana* were 90.5%, 75.39% and 80% respectively.

The present study the exponential value ‘b’ was found to be *Barbodes gonionotus* (3.8418) that expressed positive allometric growth (b > 3). Isa *et al.,* (2010) described the positive allometric growth pattern for *Puntinus* sp., in Kerian River Basin and Pedu Lake. The results of positive allometric growth which indicated that there is a good relationship between length and weight of a fish species for the growth weight pattern. That the two fish species observed (positive allometric growth) with b > 3.

In the present study, according to parabolic form, the positive correlation between total length and weight of this fish species was indicated by the value of coefficient of determination. The high value of coefficient of determination (R^2) was observed to be R^2 = 0.793 of *B.gonionotus*. The value of R^2 was close to ‘one’. Therefore, the fish species indicated to the high correlation and goodness of fit in Hinthada department of fisheries.

According to Froese (2008) the condition factor was assessed for comparisons among sexed, seasons and localities. The values of condition factors were observed to be K=1.05 of *B.gonionotus* during the study period. Le Cren (1951) stated that the value close to one was considered as good in assessing the well - being state of a fish.

The results of the present study, K value of growth pattern greater than one of the recorded fish species was examined to be expressed as the nourishment and habitat.

CONCLUSION

This paper generated some information on the early life history, embryonic and larval developmental stages, growth rates and length of *Barbodes gonionotus* that help the breeders for its propagation under captive condition. In this paper supported the researcher those who

are interested in the study of fish on embryonic and larval development, which might be of great use to take appropriate steps for the sustainable development of the culture and management technology of common carp and silver barb. It is concluded that the embryonic and larval development of recorded fish species was essential to know their history and small scale and commercial aquaculture.

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