# Induced Breeding of *Cyprinus carpio* (Linnaeus, 1758), Pond of Hinthada Department of Fishery, Hinthada Township, Ayeyawady Region

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## Abstract

*Cyprinus carpio* was selected for injection at Department of fishery in Hinthada. In the present study, the mature females and males were induced by administering the mixture of Cinnafact and Motilium at a single dose of 5.4 kg and 7.35 kg with intra - peritoneal injection. There were seven main stages of embryogenesis such as zygote, morula, blastula, gastrula, segmentation, pharyngula and hatching period. Six larval development stages were recorded in common carp such as hatching larval stage, melanoid-eye stage, yolk absorption stage, anal-caudal and pelvic fin formation stage, squamation stage and juvenile stage. Hatching was occurred in common carp at range of about 42 to 43 hours after egg fertilization. The fertilized egg appeared spherical, yellowish, transparent and 2.5 mm in diameter. The transition from larva to juvenile was occurred within 30 days. 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 *Cyprinus carpio* (4.416) that was expressed as the positive allometric growth (b>3). The value of coefficient of determination was ($R^2$ = 0.783). The value of $R^2$ was close to 'one'.

**Keywords:** induced breeding, common carp, length - weight relation and condition factor

## Introduction

Fish contain vitamins A, D, E and K which have been successfully used in controlling coronary heart diseases, arthritis, atherosclerosis, asthma, auto immune deficiency diseases and cancer (Bhuiyan *et al*., 1993 and Fasakin, 2006).

Induced spawning of local carps through hypophysation became a common practice in Bangladesh since 1967 (Ali, 1967). Meanwhile a large number of hatcheries in the private sector (estimated at over 700) have been established with the introduction of artificial breeding of exotic species (Ali, 1998). Induced spawning has opened the door of new era in the production of fish through the world. In Bangladesh, successful induced spawning was first done by Ali (1967) in carps through hypophysation having been standardized (Haque, 1975, Islam and Chowdhury, 1976 and Alam, 1983).

In 2010, it ranked third in terms of worldwide finfish aquaculture production, contributing 9% of the world's total finfish aquaculture production and Asia accounted for more than 90% of common carp's aquaculture production (FAO, 2012).

Carp is an important for food fish throughout of the world except for in Australia and North America where the fish is considered unpalatable. The world catch rate of carp per year exceeds 200,000 tons. The more colorful carp, called Koi, are bred in captivity and sold as ornamental pond fish (Banarescu and Coad, 1991; Mc Crimmon, 1968).

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*Cyprinus carpio* is one of the freshwater fish species in the family Cyprinidae. It has been used in aquaculture almost throughout human history, being cultured in China since at least 475 BC. It is a native of central Eurasia, from where it was spread by humans through Europe and much of Asia, and now is established on all continents except Antarctica - it can be considered as the world´s most widely distributed freshwater fish. It is one of the most introduced fish, ranking as the third most introduced species in the world. It is important in many parts of the world and continues to be used both in pond and captive fisheries because of its potentially rapid growth in eutrophic waters and ability to tolerate adverse environmental conditions (ISSG, 2021).

The most common form of LHRHa is the buserelin acetate. This is sold as a drug for human usage under the trade named Cinnafact. Cinnafact has been used successfully in the spawning of a wide range of fish species in many countries. Cinnafact is sold in bottles containing 10 $cm^3$ of dissolved hormone. When using Cinnafact to induce fish spawning it is necessary to mix it with domperidone maleate. Motilium comes in tablet form, with each tablet containing 10 mg of domperidone maleate. When using Motilium, it is necessary to crush the tablet into a powder and dissolve with distilled water so that it can be mixed with Cinnafact for injection into the fish (Meenakarn and Funge - Smith, 1998).

The importance of determining length-weight relationship (LWRs) in fish has been emphasized by many studies. It provides information about the growth pattern, general health, habitat conditions, life history, fish fatness and condition, as well as morphological characteristics of the fish (Schneider *et al.*, 2000; Froese, 2006).

The condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren,1951). Present study was conducted with the following objectives:

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

## Materials and Methods

### Study area and study site

The present study was conducted in Department of Fishery, Hinthada Township, Ayeyawady Region. It is located at latitude 17˙ 39′ 22″ N and longitude 95˙ 25′ 16″ E (Fig.1).

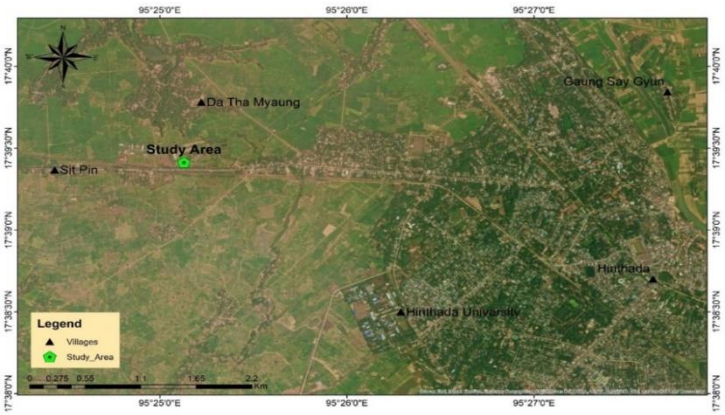



Figure 1. Location Map of the study area (Base map - Setellite Image)

**Materials using for induced breeding**

Medicinal hormones such as Cinnafact and Motilium were used for injection. Some materials such as distilled water, syringes, dissecting microscopes, digital balances and drag nets were also used during the artificial induced breeding (Plate 1).

A. Drag net

B. Digital balance

C. Dissecting microscopes

D. Syringes and distilled water

E. Concentrate spawning fish hormone (Cinnafact)


F. 10 mg - film coated tablets (Motilium)

Plate 1. Materials used in induced breeding

**Ponds and tanks for culture**

Brood stock pond, mating tank, larval tank and nursery ponds were used to culture in the study site. Some concrete tanks such as breeding tank and rearing tanks were used during the induced breeding of fish in the study (Plate 2).

A. Brood stock pond

B. Mating tank

C. Larval tank

Plate 2. Ponds and tanks for rearing fish in the study site

**Selection for spawning**

*Cyprinus carpio* were collected to breed for spawning. The fish species was taken by drag net from brood stock pond. A total of ten males and five females were chosen as spawners for one batch.

**Preparation of medicinal hormone and injection for spawning**

The breeder females were given injection of Cinnafact 1ml per kg, Motilium 5mg per kg and distilled water 1 ml 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.

**Breeding**

Injection was carried out in the evening for common carp in the study area. After injection, breeder fishes were kept into concrete tank separately by spraying water to stimulate for mating at ambient temperature 23-24˚C. Mating started about six hours after injection in common carp.

After mating, about two hours in common carp, the fertilized eggs were deposited from the female and then the eggs transferred to the other concrete tank with water hyacinth plants. After three days, the eggs become to larva stage and fry stage was occurred and transferred to the hatching 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, counted by visual observation and recorded to estimate the hatching rate. And then, survival rate the number of live fries was 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.

Samples of eggs were taken prior to hatch at every 15 minutes intervals were taken for further studies. Developmental time from fertilization was recorded 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 with drag net 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 recorded to the nearest gram with a digital balance after removing the adhered water and other particles from the surface of the body.

**Calculation of length -weight relationship**

The following method was adopted for the assessment of various measurements of parameters and their 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 equation.

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.

## Results

Selections of breeding sets were constructed as two males and one female. The total weight of 7.35 kg of males and 5.4 kg of females was chosen for *C. carpio* during the study period.

**Deposition of eggs and production of fries**

In common carp, the ovulation time lasted from six to eight hours in ambient temperature 23˚C. In three plants of water hyacinths (0.276kg) and hundred plants of water hyacinths (4kg) received 86,004 eggs and 2,866,800 eggs. Fertilized eggs were spherical, yellowish and transparent. But unfertilized eggs were opaque and whitish. In petri dish, 100 eggs were observed 88 fertilized eggs and in 82 fries were survived 31 fries. And then, eggs were directly taken into pond after hatching in ambient temperature 24˚C (Plate 3).

A. Weighting of water hyacinth

B. Counting of eggs

C. Larvaes

Plate 3. Deposition of eggs and production of fries

**Fertilization, hatching and survival rates**

The performance of breeders subjected to induce spawning during this investigation. In common carp, the average fertilization rate of the eggs, hatching rate of fertilized eggs and survival rates of fries were obtained 88%, 93.2% and 37.8% at the ambient temperature 23℃ (Table 1).

Table1. Spawning and hatching performances of common carp

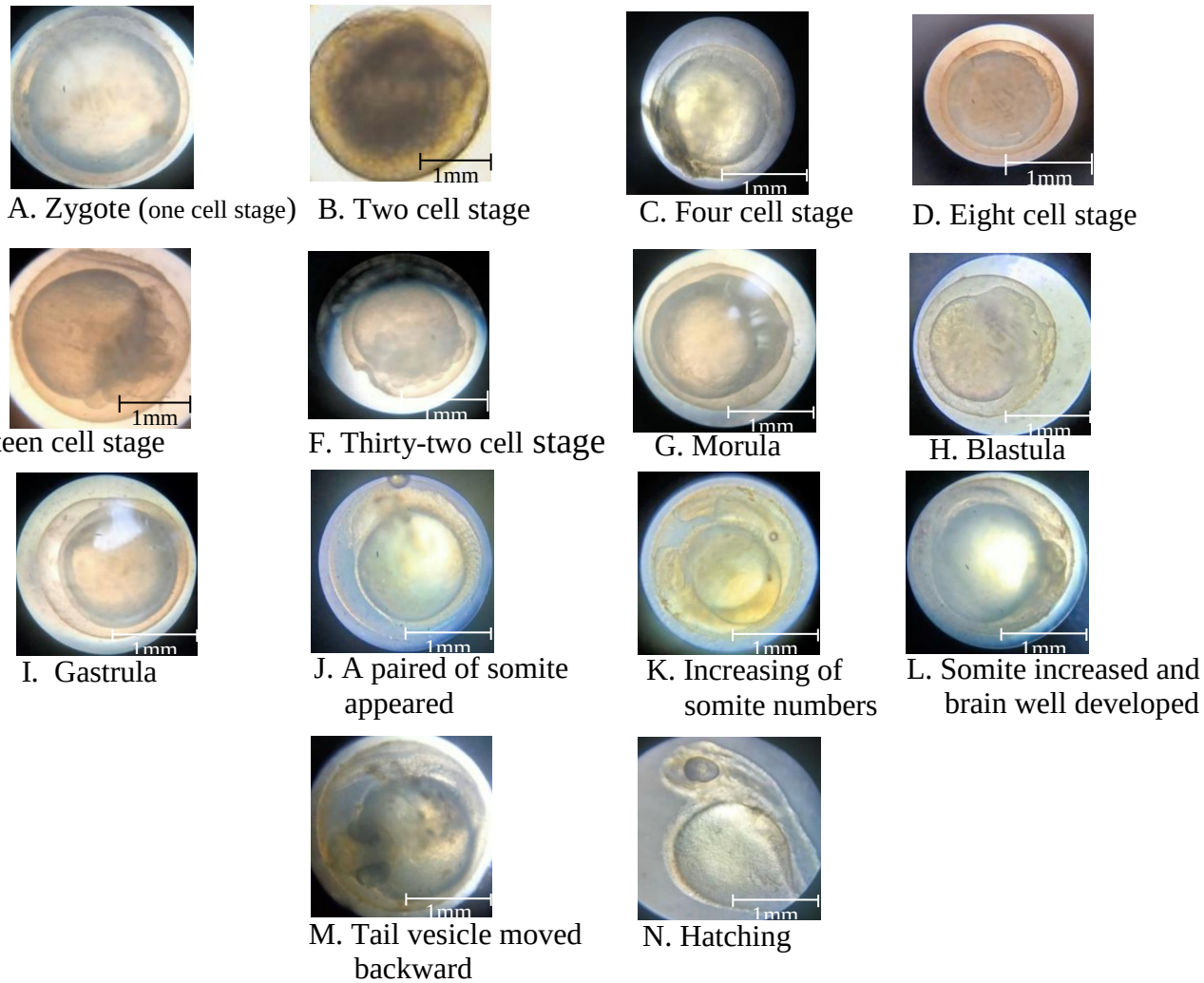
| No. of eggs | No. of fertilized eggs | No. of hatchlings | No. of survival fries | Fertilization rate | Hatching rate | Survival rate |
|---|---|---|---|---|---|---|
| 2,866,800 | 2,522,784 | 2,350,776 | 888,708 | 88% | 93.2% | 37.8% |

**Developmental stages**

In general, the development of common carp 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 stages: zygote (including cleavage), morula, blastula, gastrula, segmentation, pharyngula and hatching period (Plate 4).




A. Zygote (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

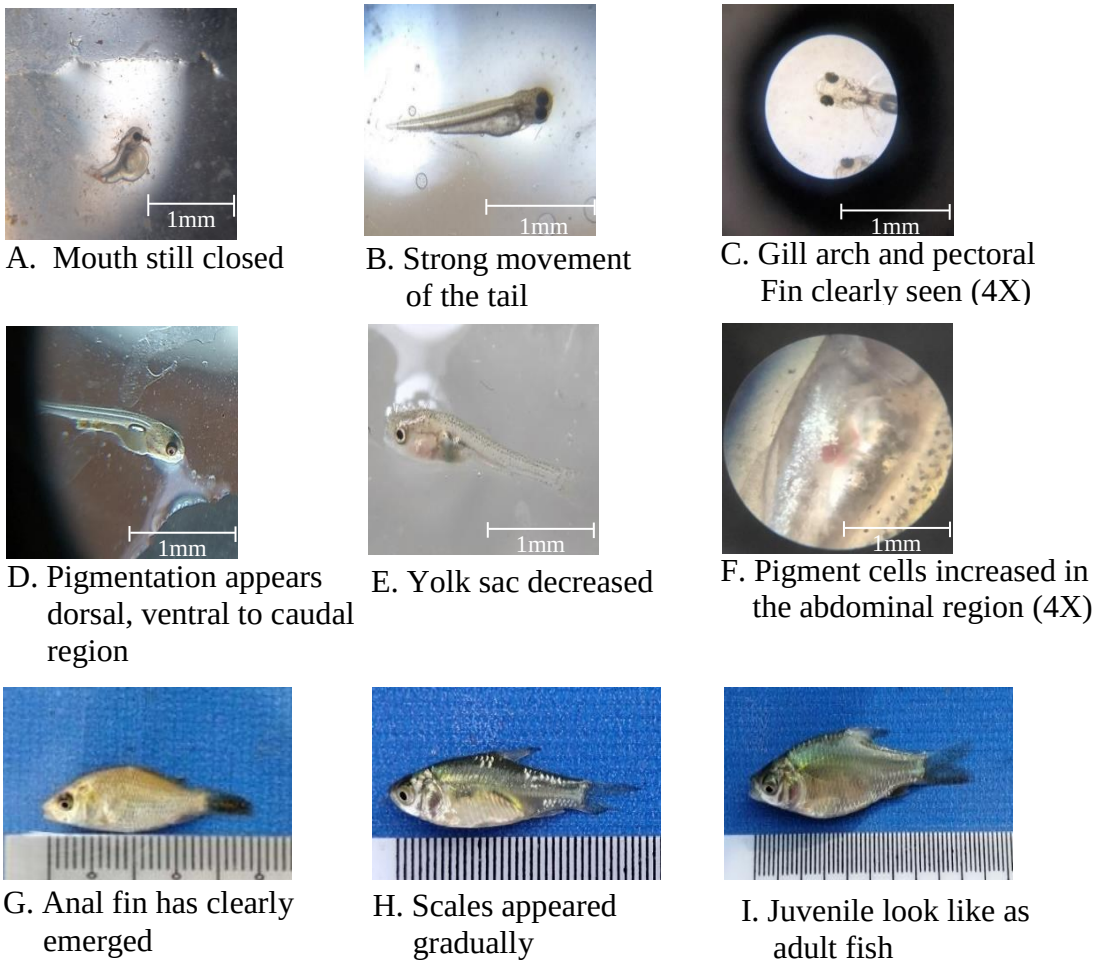
I. Gastrula J. A paired of somite appeared K. Increasing of somite numbers L. Somite increased and brain well developed

M. Tail vesicle moved backward N. Hatching

Plate 4. Development stages of hatching (4X) in *Cyprinus carpio*

**Larval stages**

Six developmental stages were distinguished in the common carp larvae, starting from the hatching larval stage to the juvenile stage (Plate 5).




A. Mouth still closed
B. Strong movement of the tail
C. Gill arch and pectoral Fin clearly seen (4X)
D. Pigmentation appears dorsal, ventral to caudal region
E. Yolk sac decreased
F. Pigment cells increased in the abdominal region (4X)
G. Anal fin has clearly emerged
H. Scales appeared gradually
I. Juvenile look like as adult fish

Plate 5. Development of post hatching larval stages in *Cyprinus carpio*

**Relationship between body weight and total length**

The thirty individual were observed with the measurement and body weight to calculate the relationship between body weight and total length. The regression analysis was calculated the length-weight relationship for common carp 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 were obtained the value b = 4.4169 and (> 3), the growth rate is considered as positive allometric.

$R^2$ and ‘r’ values of total length and body weight were 0.783 and 0.88 respectively. The value of condition factor ‘K’ was found to be 1.03 (Table 2 and Fig .2).

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

| Sr. No | Length(X) | Weight(Y) | Log of length | Log of weight | XY | X^2 | Y^2 | a L^ b | Kvalue |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.2 | 0.2 | 0.505 | -0.698 | -0.353 | 0.255 | 0.488 | 0.327 | 0.610 |
| 2 | 3.5 | 0.4 | 0.544 | -0.397 | -0.216 | 0.296 | 0.158 | 0.486 | 0.821 |
| 3 | 3 | 4 | 0.9 | 0.602 | -0.045 | -0.027 | 0.362 | 0.002 | 0.878 |
| 4 | 4 | 3 | 0.1 | 0.477 | -1 | -0.477 | 0.227 | 1 | 0.246 |
| 5 | 5 | 3.5 | 0.4 | 0.544 | -0.397 | -0.216 | 0.296 | 0.158 | 0.486 |
| 6 | 6 | 3.5 | 0.4 | 0.544 | -0.397 | -0.216 | 0.296 | 0.158 | 0.486 |
| 7 | 7 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 |
| 8 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 | 1.026 |
| 9 | 3.5 | 0.6 | 0.544 | -0.221 | -0.120 | 0.296 | 0.049 | 0.486 | 1.232 |
| 10 | 3.2 | 0.4 | 0.505 | -0.397 | -0.201 | 0.255 | 0.158 | 0.327 | 1.220 |
| 11 | 4 | 1.1 | 0.602 | 0.041 | 0.024 | 0.362 | 0.001 | 0.878 | 1.252 |
| 12 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 | 1.026 |
| 13 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 | 1.026 |
| 14 | 3.5 | 0.4 | 0.544 | -0.397 | -0.201 | 0.296 | 0.158 | 0.486 | 0.821 |
| 15 | 3.2 | 0.4 | 0.505 | -0.397 | -0.201 | 0.255 | 0.158 | 0.327 | 1.220 |
| 16 | 5 | 1.7 | 0.698 | 0.230 | 0.161 | 0.488 | 0.053 | 2.353 | 0.722 |
| 17 | 4.2 | 1.1 | 0.623 | 0.041 | 0.025 | 0.388 | 0.001 | 1.089 | 1.009 |
| 18 | 3.5 | 0.7 | 0.544 | -0.154 | -0.084 | 0.296 | 0.024 | 0.486 | 1.437 |
| 19 | 3.5 | 0.7 | 0.544 | -0.154 | -0.084 | 0.296 | 0.024 | 0.486 | 1.437 |
| 20 | 3.2 | 0.4 | 0.505 | -0.397 | -0.201 | 0.255 | 0.158 | 0.327 | 1.220 |
| 21 | 5 | 2 | 0.698 | 0.301 | 0.210 | 0.488 | 0.090 | 2.353 | 0.849 |
| 22 | 4.2 | 1 | 0.623 | 0 | 0 | 0.388 | 0 | 1.089 | 0.917 |
| 23 | 4 | 0.9 | 0.602 | -0.045 | -0.027 | 0.362 | 0.002 | 0.878 | 1.024 |
| 24 | 4 | 0.8 | 0.602 | -0.096 | -0.058 | 0.362 | 0.009 | 0.878 | 0.910 |
| 25 | 3.5 | 0.4 | 0.544 | -0.397 | -0.216 | 0.296 | 0.158 | 0.486 | 0.821 |
| 26 | 3.5 | 0.8 | 0.544 | -0.096 | -0.052 | 0.296 | 0.009 | 0.486 | 1.642 |
| 27 | 4 | 1.3 | 0.602 | 0.113 | 0.068 | 0.362 | 0.013 | 0.878 | 1.480 |
| 28 | 3.2 | 0.4 | 0.505 | -0.397 | -0.201 | 0.255 | 0.158 | 0.327 | 1.220 |
| 29 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 | 1.026 |
| 30 | 3.5 | 0.5 | 0.544 | -0.301 | -0.163 | 0.296 | 0.090 | 0.486 | 1.026 |
| | 109.9 | 20.5 | 16.818 | -7.175 | -3.664 | 9.510 | 3.737 | | 1.03 |

b nExy minus ExEy (-109.923 - -120.680) = 10.7567

nEx2 minus Ex) ^2 (285.301 - 282.866) = 2.4353= 4.41688b

a Ey minus bEx (-7.175 - 74.2845) = -81.4579

n = -2.7152 = 0.001926 a

antilog of obtained a value

K =W/W^

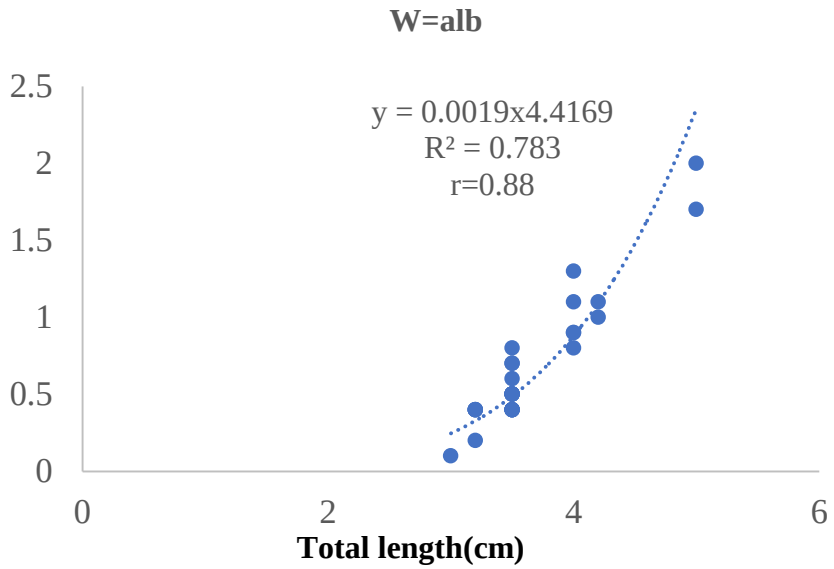



Figure 2. Parabolic form of common carp

## Discussion

Go Sian Siam (2021) reported that the hatching stage observed in common carp at 24-38 hours after spawning in water temperature 25˚C by using Suprefact and Motilium Tablet. The two cells, four cells and eight cells stages were found within 45, 65and 80 minutes after fertilized respectively. Both the fertilization rate and hatching rate were about 77%. In present study, of the common carp, the hatching stage were observed at 42 - 43hours after spawning in ambient temperature 23˚C by using Cinnafact and Motilium Tablet. The same series of cleavage were observed at 80, 100 and 155 minutes after fertilization. Fertilization, hatching and survival rates were 88%, 93.2% and 37.8%. When comparing between these studies, different points was found because cleavage time, fertilization and hatching rate affected by variation of temperature, physical condition and type of hormone using in the brood fishes.

Morula stage in present study was found in 3:40 hours after fertilization where Ghosh *et al.,* (2012) observed the same stage in koi carp 5:10 hours after fertilization and Haniffa *et al.,* (2006) observed the same stage in koi carp 4:40 hours after fertilization. These might be due to temperature and species difference.

Nica *et al.,* (2012) found that hatching in koi carp occurred 50 to 58 hours after spawning in water temperature 22˚C whereas Haniffa *et al*., (2006) observed the hatching stage in koi carp 32 to 34 hours after fertilization in ambient temperature 26 - 28˚C. In the present study, the same stage was found in 42 to 43 hours after fertilization in ambient temperature 24˚C. The two cells, four cells and eight cells stage were found within 80,100 and 155 minutes after fertilized respectively. According to Nica *et al.,* (2012) in koi carp, the cleavage was observed at 40, 50 and 95 minutes after fertilization. Second day later; the hatchlings swam freely and by third day the hatched larva mouth formation complete and started feeding. In koi carp, the same series occurred at 60, 90 and 110 minutes after fertilization, Haniffa *et al.,* (2006).

Balon (1995) and Haniffa *et al*., (2006) found more or less similar in case of common carp and koi carp. The fertilized eggs were round, transparent, demersal and adhesive. The color of the fertilized eggs was yellowish white.

Miah *et al*., (2008) found that the difference of the egg diameter was due to the species and brood size of common carp. Kuo *et al*., (1973) and Liao (1975) also reported that the incubation period of carp's eggs and larval development would depend largely on water quality parameters such as salinity and temperature.

In present induced culture, the sex ratio was 2:1 (male and female) and the doses of medicine with the mixture of Cinnafact, Motilium and distilled water 12µ/kg, 5mg/kg and 1CC/kg were used for each female. The hatching period was observed 29:25 to 42:50 hours at the ambient temperature was 23℃. The average fertilization, hatching and survival rates were 88%, 93.2 % and 37.8% respectively.

In the present study, the value of 'b' was found to be (4.4169) that expressed positive allometric growth (b > 3). The value of 'b' greater than 3 also suggests a healthy environment for the common carp to feeding and growth. Singh *et al*., (2015) recorded positive allometric growth (b = 3.097) for common carp.

The high values of coefficient of determination ($R^2$) was observed to be $R^2 = 0.783$. The value of $R^2$ was close to 'one'. Therefore, the fish species was indicated to the high correlation and goodness of fit in department of fisheries.

The value of condition factor was observed to be K=1.03 during the study period. Le Cren (1951) stated that the value close to one is considered as good in assessing the well - being state of a fish. The K value was obtained in the present study was greater than one which suggests that the fish in a good condition.

## Conclusion

The results indicated that injection of common carp with using Cinnafact and Motilium was more effective in induction of ovulation, increasing fecundity and hatching rate. The successful induced breeding of this fish may increase the large scale production for export purpose. And then a potential sector for meeting the national demand and help to increase foreign exchange earnings.

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