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A Study on Reproductive Behavior and Fry Nursing of Koi Carp (*Cyprinus rubrofuscus*) in Sunsari, Nepal

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ABSTRACT

A study was conducted emphasizing, the induced breeding techniques, breeding behavior and nursery rearing of koi carp at Everest Aquaponics and fish farm, Sunsari, Nepal. The study was done in the hatchery Unit of EAFF for 36 days including 28 days of fry nursing from 15 May to 26 June. Ten set of brooders in the ratio of 1:2 was used. The average body weight of the female brooders was 910±164.722 gm and a female weighing one kilogram gives approximate 70,219.78 eggs in induce breeding method by hand stripping. The percentage of average fertilization rate was 68.6±3.58 %, the average hatching rate was 75.75 ± 5.9% and average survival of spawn was 66.88±5.84%. The size of the fry after hatching also varied between 2.3-3 mm whereas the fry growth varied from 17- 24mm and the average survivability from fry to fingerling was 88.9±1.59%. The mean final weight of the fry in the nursery tank was 1.0845±0.0353 gm and the average weight gain after 28 days of nursery rearing was 0.9315gm±0.013298gm. The average of the water quality values including temperature was 27.5°C, pH was 7.975, dissolved oxygen was 6.55 mg/L, NO_3 concentration was 3.75 mg/L during nursery raising whereas the average water parameters during breeding were 26.75°C, pH of 7.995, DO of 6.25 mg/L, NO_3 of 4.375. Successful koi farming can reduce the uncertainty and unavailability of fingerlings, can increase large-scale production for export, and can be a potential area to meet national needs and help increase revenue in foreign currencies. Current research will certainly pave the way for startups to launch commercial production and commercialization of Koi carp in the coming days.

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. As a fish hobby, ornamental fish keeping has travelled across the boundaries of nation, generating potential for cultural exchange. It has been identified as the oldest and second most popular hobby in the world next to photography (Ghosh *et al*., 2003). The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally. This trade has a turnover rate of over 5 billion USD and annual growth rate of over 10% with Asia and pacific still being the highest exporting parties. (Ghosh, 2012; Agbayani *et. al,* 2021; WIRE, 2021)

Induce breeding of exotic carp such as common carp and native carps have been established as a dependable source of seed since mid-1960s and 1970s respectively. This was one of the major breakthroughs in the development of aquaculture in Nepal (National aquaculture sector overview, FAO, 2022). In Nepal, the trade of ornamental fish is confined to a small territory till now. Ornamental fish breeding and nursing technologies of only six exotic fish including one native ornamental fish species have been developed at fishery research station (FRS, Pokhara; Husen, 2019). In order to sustain growth in overall aquaculture of Nepal, it is absolutely necessary to shift the focus on breeding of ornamental fish in private sectors. Organized trade in ornamental fish depends on assured and adequate supply and demand, which is possible only by mass breeding (FAO,2022; Ghosh, 2012).

Koi Carp are colorful, ornamental fish belonging to magoi carp / Amur carp (cyprinus rubrofuscus) species native to East Asia (History of Nishikigoi, 2021). The word koi is derived from Japanese word "Nishikigoi". The word "Nishiki" means beautiful (or elegance) traditionally in Japan whereas koi is a homophone for word "affection" or "love" in Japanese language (Kodama, 2021). Koi belongs to cyprinidae family. The systematic breeding of ornamental koi in Japan began in Ojiya and Yamakoshi in the Niigata prefecture in the 1820s (koshida, 1931; kodama, 2021). There are easily over 100 varieties of koi with new types being developed actively. According to Zen Nippon Airinkai , Koi Carps are categorized under the basis of their coloration, pattern and body confirmation and are classified into following recognized classification i.e. Kohaku, Taisho sanshoku, Showa sanshoku, Bekko, Utsurimono, Asagi, Shusui, Koromo, Goshiki, Kawarimono, Kinginrin, Hikari Utsuri, Hikari moyo, Hikari muji, Tancho, Chagoi, Ogon, Kumonryu, Kikokuryu, Ghost koi, Doitsu koi, Butterfly koi. Among them: Kohaku, Taisho Sanshoku (sanke) and Showa Sanshoku (showa) are considered true Nishikigoi.

The color and scale pattern of this species is highly variable. They grow up to 100 cm living more than 20 years in their natural habitat (Kuroki, 1981). Males live longer than females. Carps can mature at the age of 3 months and some at 5 years of age (Fernandez, 1990). This species exhibits external fertilization, with spawning frequency that varies throughout their range (Balon, 1990) and are considered as batch spawnners. They are highly fecund species, typical relative fecundity ranges

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from 1, 00,000 to 3, 00,000 oocytes per kg total body weight (Hanchet, 1990). The marketable size could be achieved within 2-6 months depending upon the size demand under conventional aquaculture condition. Considering the enormous importance of koi carp in Nepalese ornamental fish market and world ornamental fish industry, this study was conducted emphasizing the induced breeding techniques, breeding behavior and nursery rearing of koi carp at EAFF, Sunsari, Nepal to address the existing problem, propose a set of attainable goals with the aim to establish cohesive production cycle of koi carp in Nepal.

MATERIALS AND METHODS

Experiment Site

Two consecutive experiments were conducted to assess the breeding performance, survival and growth of koi carp fry between 15 may and 26 June, 2021 at Everest Aquaponics and fish farm (EAFF). It is located in Chakarghatti, 25km southwest to Dharan, Nepal at latitude 26.7326° N and longitude 87.1323° E. The monthly minimum and maximum temperature vary between 17 degrees to 32 degrees throughout the year.

Brood Fish Collection and Sex Determination

Koi fish were collected from the broodstock pond and transported to the hatchery units. Male and female of at least one and half year are selected for breeding with body weight on minimum 700gm They were stocked and acclimatized in a brooder tank (4.5x3x1m3) from hatchery unit for 5 Days. Same variety of breeding pairs was selected to have precise control over breeding. The brood stock would be checked regularly based on external morphological features associated to ripeness, e.g., swollen reddish genital papilla, soft abdominal region, and comparatively large size. Female gets distinguished from male as they have rounder body shape with slimy coating all over the body whereas males are relatively cylindrical with thin body. The males will also have classic breeding tubercles on their gill plates and pectoral fins during the breeding season.

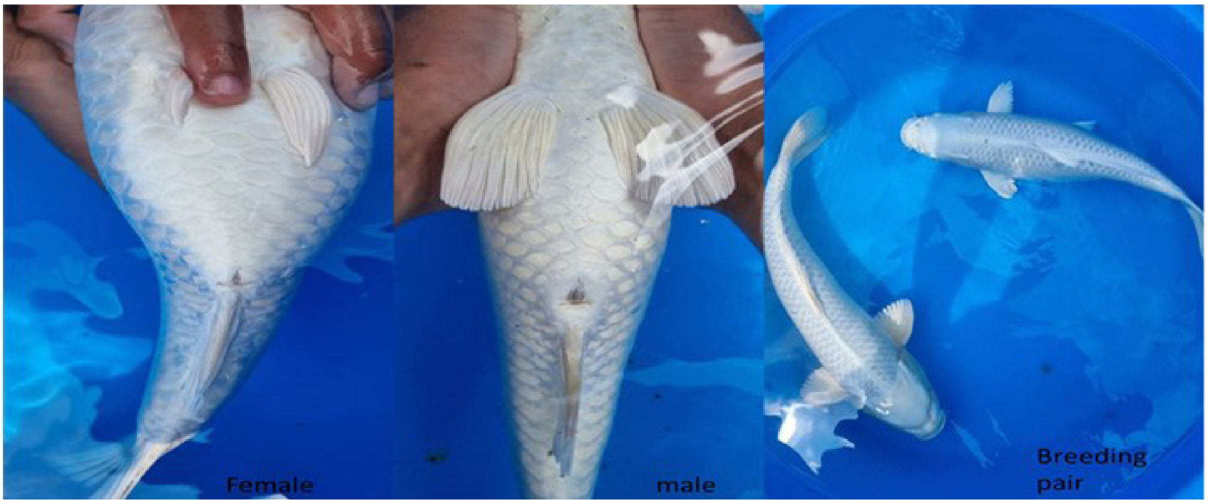

Figure 1: Female and Male genital openings in koi fish for sex determination**.**

Brooders Nursing and Conditioning

High nutrition value feed such as pellets, egg yolk, daphnia and jelly foods (sometimes) were given to the brooders for 15 days. Male fish and female fish were kept in a different tank to avoid natural spawning. They were fed with CPF floating pellets 7% of their total biomass with 32% of crude protein 3 times (10 am, 2pm, 5pm) a day (FAO, 2022). Jelly food containing beef heart, egg yolk, crumbled pellets, essential vitamins and algae wafers was made and fed sometimes to diversify the diet and nutrition supply for the brooders. 50 percent water change was done once in 3 days and the brooder fish were fed with live fish such as Moina and other crustaceans for proper maturity of gametes. The brooders were sometimes fed up to 5 times in warmer days and least a time in rainy days.

Detail of Experiment

The research was carried out in a completely randomized design (CRD) with 10 experimental sets with no replication giving total of 30 experimental units. In this study, 20 male and 10 female brood fish are used for breeding. Sex ratio of 2:1 i.e., 2 males for a female was kept. The brooders were selected on the basis of lineage as same color patterned brooders are used. The brooders were injected with spawn pro hormone on the basis of their body weight. They were left in a hatchery pond. The brooders tend to show mating signs early at the morning. These brooders were now selected and stripped by hand in a tub. After stripping each brooder, sampling techniques was used to collect required data for breeding and water quality parameters and fries were then transferred to nursery grow out ponds for growth monitoring activity.

Hormone Administration

Required dose of hormones were injected through syringe below the starting portion of the dorsal fin. Male fish was given one injection of Ovaprim at the rate of

0.2ml/kg body weight. The females were injected with 0.5ml Ovaprim per kg of body weight. The dose is calculated based on the weight of the fish i.e.
Fish Weight X Ovaprim Dosage= Amount of Injection (FDA, 2017).

Fertilization

Before artificially stripping females and males, their weight was taken and tagged. A smaller "indicator" male was added, the ripest females would start to simulate spawning with it. After 15-20 minutes of first spawning splashing, ovulation was completed hence, the stripping of ripe females was done 14-16 hours after the first injection at temperature range of 25-28 ^{0}C.) The eggs obtained from stripping were weighed, followed by mixing several drops of creamy milt squeezed over the eggs with dry hands. Milt of two males for one female was used (10ml sperm for 1 kg of eggs. A 0.9% (NaCl) saline solution was added, causing gentle movement of sperms. The process aids to fertilization. Eggs were spread on a screen of 1mm mesh size, which was then placed in an incubating tank. The fecundity was calculated by using gravimetric method where sampling was done based upon weighing of eggs. The total number of eggs was then weighed where random sample of about 250 eggs were counted out and weighed. Fecundity, fertility, hatchability and survivability rate were calculated by using following formulas;

1. Fecundity= (number of eggs in the subsample X total weight of the ovaries)/ weight of the subsample in the same units
2. Fertilization rate = (number of fertilized eggs in sample/total number of sample eggs) x 100%
3. Hatching rate (%) = (no. of hatchlings/ total number of fertilized eggs) × 100
4. Survival rate (%) = (number of larvae at the end of study/ number of larvae at the starting of the study) x 100

Water Quality Requirement

Water used for the culture of fishes deteriorates rapidly and should be partially changed or refreshed frequently. The water supply was stopped during feeding to avoid food thinning. The experimental water quality parameters were monitored daily, parameters like pH, DO, nitrates level, TDS and temperature during the trials using different instruments such as ph meter, DO test kit and TDS meter.

Food and Feeding of Larvae

After the egg yolk was absorbed completely by larvae fish within 3 days, first feeding was done. Mixture of infusoria and brine shrimps was given by dissolving in water. The fry was fed with Artemia for 7 days and then take a commercial feed in a size of 0.2 mm as well as water crustaceans such as Moina and daphnia Each chamber larvae was fed uniformly at 10 % of their body weight four times a day (7 am, 12 am, 3pm, and 5pm). Larvae stomach content is checked after every feeding. Crumbled pellets with nutrient content of at least 33% protein, 4-6 % fat, 5-8 % fiber and 9-10 % moisture content were used. Feed with enough fatty acid and pigmentation should be fed for koi larvae as they have tendency to absorb or collect pigments, generally during development from the environment. This help in improving the lusture in their coloration and skin quality (Alex S, 2012).

Growth Monitoring

The growth rate of larvae samples from each brooder were monitored and recorded weekly. 250 sample larvae from each container were monitored and fed uniformly. Mean length growth and mean weight gains were recorded weekly from all 10 experimental units. The growth of the larvae was monitored for 28 days after fertilization. 50 percent water change was done once in every three days. Filter chamber with both UV sterilizer and bio filtration units was installed in the reservoir which collects the underground water.

Harvesting and Marketing

Fries that remained in the hatchery unit of EAFF were left in a mud pond for further grow out and rest of the survived hatchlings which were reared for study purpose for 28 days were sold to local whole sellers for RS.32/pc.

Statistical Analysis

Analysis of data was done using MS-Excel. Means were expressed as mean ± SD for all the parameters. Data were shown by suitable tabulation.

RESULTS AND DISCUSSIONS

Maturity Test and Breeding Behavior

The breeding experiment was conducted by induce breeding method in a captive condition by maintaining 10 sets of brood fish from 20th may to 23 may, 2021 when the average water temperature was 26.625 ± 1.19°C. The average body weight of mature female brooders used

Table 1: Average physiological and breeding parameters of Koi carp

S.N	Description	Unit	Average parameters
1.	Temperature	°C	26±1.19
2.	Female body weight	Gm	910±164.722
3.	Weight of eggs	Gm	127.8±12.53
4.	Eggs count per female	Nos.	63,900±6266.312
5.	Hatching rate	%	75.5±5.9
6.	Survival of fry out of total fertile eggs	%	66.88± 5.84
7.	Survival from larvae	%	88.9± 1.59
8.	Relative fecundity	Eggs/gm body weight	71.34±2.5

9.	Fertilization rate	%	68.6±3.58
10.	Incubation Period	Hrs	48±1.2
11.	Latency period	Hrs	16 ±1.6

for breeding was 910±164.722 gm. The average weight of eggs per female was 127.8±12.53gm. In average the total eggs spawned in was 127.8±12.53 gm. The relative fecundity was 71.34±2.5 egg/g. The fertilization rate was 68±3.58% in induce breeding of koi carp. The hatching rate of the fries from fertile eggs was 75.5±5.9%. The latency period in the induced breeding was 16 ±1.6 hrs. The incubation period was 48±2hrs.

Fecundity

The ovulation rate treated with synthetic hormone is shown in Table 2. The highest ovulated eggs were 75000 from a brooder of 980 gm body weight whereas the lowest fecundity was 54,000 eggs from a brooder of 690gm. The average mean fecundity was 63,900 ±6266.312 and mean fecundity per kg female was around 70,219.78.

Table 2: Fecundity of Brooder Koi

Tank	Weight before(gm)	Weight (after stripping or breeding) (gm)	Fecundity weight (gm)	Number of egg counts
	1230	1086	144	72,000
2	690	582	108	54,000
3	900	768	132	66,000
4	720	602	118	59,000
5	980	830	150	75,000
6	870	740	130	65,000
7	820	702	118	59,000
8	950	828	122	61,000
9	1100	969	131	65,500
10	840	715	125	62,500
Mean	**910±164.722**	**782.2±155.3**	**127.8±12.53**	**63,900±6266.312**

Fertilization Rate

The fertilization rate of brood fish injected with synthetic hormone spawn pro was in the range of 63.6- 75.2% out of 250 eggs samples kept in 10 different tanks. The average fertility rate was 68.6±3.58%. Tank 9 had the highest fertility rate of 75.2% while the lowest fertility rate was 63.6% where only 171 eggs were fertile out of 250 fecund eggs.

Table 3: Fertility rate of Koi carp out of 250 sample eggs

Tank	Number of fertile eggs	Fertility Rate (%)
1	178	71.2
2	161	64.4
3	178	71.2
4	167	66.8
5	176	70.4
6	171	68.4
7	159	63.6
8	173	69.2
9	188	75.2
10	164	65.6
Mean	**171.5±8.95**	**68.6±3.58**

Hatching Rate

Significant hatching rates (87.57%) were recorded in tank 2 where the number of eggs hatched from 161 fertile eggs was 141. The average hatching rate was 75.75 ± 5.9% with 87.57% and 70.52% being highest and lowest hatching rate from the given table respectively.

Survival rates

The survived fry was calculated at the end of 28 days. Dead fries were counted every day. From the present study result, the lowest survival rate of fry was 87% and highest survival rate was 91% with an average of 88.9±1.59%. The average survival rate of fry from fertilized eggs was

Table 4: Comparison of hatched eggs, number of mortality and survived eggs

Tank	Number of eggs (fertile)/250eggs	Number of hatched eggs	Hatching rate (%)
1	178	129	72.4
2	161	141	87.57
3	178	126	70.78
4	167	121	72.45
5	176	135	76.7
6	171	123	71.92
7	159	120	75.47

8	173	122	70.52
9	188	159	84.57
10	164	119	72.56
Mean	**171.5±8.95**	**129.5±12.5**	**75.5±5.9**

Table 5: Comparison of Survivability of Koi carp with different breeding parameters

S.No.	**Survived fries**	**S~F %**	**S~H%**	**S~f%**	**Mortality rate%**
1	114	66.28	89	45.6	11.63
2	128	79.50	91	51.2	9.22
3	109	61.24	87	43.6	13.49
4	108	64.67	90	43.2	10.74
5	120	68.18	89	48	11.11
6	107	62.57	87	42.8	12.90
7	105	66.04	88	42	12.50
8	109	63.01	90	43.6	10.66
9	140	74.47	91	56	11.95
10	103	62.80	87	41.2	13.45
Mean	**114.3±11.75**	**66.88±5.84**	**88.9±1.59**	**45.72±4.7**	**11.76±1.37**

(Note S= survivability, F = fertility, H= hatchability, f= fecundity)

66.88±5.84%. The average mortality rate at the end of the study was 11.76±1.37% with highest and lowest mortality rate being 13.49% and 9.22% respectively.

Growth Performance

The growth performance of the koi fish fry from the day of hatching i.e., 26th may, 2021 in treatment tanks to 24 June, 2021 was recorded in terms of length and weight.

Growth performance in length

The length of the 10 hatchling was measured on the day of hatching from hatched eggs. Length of the fry was measured from all treatment tanks on weekly basis. Each 10 fry were randomly measured and their growth in length was recorded. The study was carried out from 26th may to 24 June, 2021 in grow out tubs. The mean initial length of fry was 2.715 ± 0.047mm and the mean final length of fry after 28 days of nursery rearing was 20.65±0.944 mm. the average length gain was 17.935mm at the end of experiment. The length gain per day was 0.64mm per day whereas the specific growth rate in length was 23.5% per day.

Table 6: Growth rate of Fries

Length of fries (mm)					
Tanks	**26 May**	**3 June**	**10 June**	**17June**	**24June**
T1	2.3-3	6-6.5	6.8-7.4	13-15	19-23
T2	2.5-3	6-6.6	6.8-7.5	13-16	18-22
T3	2.5-3	6.1-6.5	6.9-7.3	12-15	18-21
T4	2.5-3	6-6.5	6.7-7.4	13-16	19-22
T5	2.5-3	5.9-6.6	6.7-7.3	12-16	17-22
T6	2.3-3	6.1-6.6	6.8-7.5	12-16	20-24
T7	2.3-3	6-6.6	6.7-7.5	13-16	20-23
T8	2.5-3	5.9-6.5	6.7-7.5	12-15	20-24
T9	2.4-3	6.1-6.5	6.8-7.4	13-16	19-22
T10	2.5-3	6-6.5	6.9-7.5	12-15	18-22
Mean	**2.715±0.047**	**6.275±0.042**	**7.105±0.055**	**14.05±0.437**	**20.65±0.944**

Table 7: Growth in weight of fries for 28 days

Weight of fries (gm)					
Tanks	**May 26**	**3 June**	**10 June**	**17 June**	**24 June**
T1	0.151	0.32	0.39	0.655	1.055
T2	0.155	0.33	0.42	0.675	1.11
T3	0.152	0.32	0.40	0.66	1.075
T4	0.152	0.31	0.411	0.65	1.05
T5	0.152	0.32	0.40	0.63	1.055
T6	0.155	0.33	0.41	0.675	1.12
T7	0.153	0.33	0.40	0.65	1.15
T8	0.155	0.33	0.41	0.66	1.07
T9	0.153	0.32	0.42	0.655	1.05
T10	0.152	0.32	0.41	0.67	1.11
Mean	**0.323±0.0067**	**0.4071±0.0095**	**0.658±0.013**	**1.0845±0.0353**	**0.153±0.001**

Growth Performance in Weight

The growth parameters in terms of weight were studied for 28 days in nursery rearing tanks. 10 Sample fry weight from each nursery tank was measured and recorded for 28 days on weekly basis from 26 May to 24 July. The growth parameters of hatched fry in the nursery tank fed with boiled eggs, artemia, crustaceans like moina and crumbled pellets during the grooming phase in nursery tanks is presented in the table. In the above table, the initial mean weight of the fry during hatching was 0.153±0.0014gm and the mean final weight of the fry in the nursery tank on 24 July, 2021 was 1.0845±0.0353 gm. The daily weight gain was 0.033gm per fish per day and average weight gain after 28 days of nursery rearing was 0.9315 gm.

Table 8: Growth parameters of fries in nursey unit

S.No	Parameters	Values
1	Mean initial weight (gm)	0.153±0.0014
2	Mean final weight (gm)	1.0845±0.0353
3	Culture Days	28
4	SGR (%/day)	23.5%
5	DWG (gm/fish/day)	0.033
6	Weight gain (gm)	0.9315
7	Mean Initial length (mm)	2.715 ± 0.047
8	Mean Final length (mm)	20.65±0.944
9	Length gain (mm)	17.935
10	Survival rate (%)	88.9±1.59

Physiochemical Parameters

Water quality parameters during breeding

Table shows the daily average temperature, DO and pH, TDS, NO3 concentration of the breeding chambers. The temperature in the breeding chamber ranged from 21-33°C with lowest on 23rd May and highest on 21st may. The averages DO in the breeding chamber ranged between 5-8 mg/L with lowest on 21st July and highest on 22 July. Similarly, the pH in the breeding chamber ranged between 7.68-8.33 with lowest on 20th July and highest on 23rd July. In the same way, the ammonia concentration in the breeding chamber ranged from 2.5 – 5 mg/L with lowest concentration in 20 May. The TDS in the chamber ranged from 71 to 142 with highest conductivity in 23rd May and lowest in 20th May.

Table 9: Growth parameters of fries in nursey unit

S. NO	Water quality parameters	Unit	Average value
1	Temperature	°C	26.75±1.190238
2	pH		7.9975±0.320559
3	Dissolved Oxygen (DO)	Mg/L	6.25±1.258306
4	NO3 Concentration	Mg/L	4.375±1.25
5	Total Dissolved Solid	Ppm	99.25±30.44531

Water quality parameters during nursery raising

The table shows the average temperature, DO and pH and other parameters of the nursery tanks taken on a weekly basis during 28 days of fry rearing time from 26th may to 24 June, 2021 in the hatchery unit of EAFF. During study period water quality parameters were measured and recorded.

Table 10: The average water quality parameters measured at different nursery tanks over 28 days during induced breeding

Water quality parameters	Unit	Values
Temperature	ºC	27.5±0.577
Dissolved oxygen	mg L^{-1}	6.55±0.310
pH	-	7.9765±0.044
TDS	µs cm^{-1}	79±26.54
NO3 concentration	mg L^{-1}	3.75±0.244

Temperature

Temperature was measured every day and there was slight fluctuation during the study period. The value was in the ranged between 23.25-31.75 ºC in overall treatments. The mean temperature was 27.5±0.577 ºC. During the experiments, the difference between water temperature changes from 21- 32ºC in breeding tanks and 22- 33 ºC in nursery tanks, which falls above than recommended values. According to Watson *et al*, water temperature between 18 to 24 °C is suitable for Cyprinus rubrofuscus rearing and growing (Watson *et al*, 2004). The results from El-gamal indicated that the optimum percent of healthy eggs was 77% at temperature of 27°C, followed by 59% at temperature of 30C°(Hakim & Gamal, 2009). Average Water temperature in the breeding tank can be considered good for breeding because the water temperature between 26-27°C is considered good for breeding (Ghosh *et al*, 2012). Although the temperature went high up to 32 degrees in the afternoon, it had very less significance to the breeding of the koi as they tend to mate in morning time.

Table 11: Mean temperature range in nursery tanks from week 1 to week 4 of raising period

Temperature Week	Minimum (°C)	Maximum (°C)	Average (°C)
1	23	31	27
2	22	32	27
3	23	33	28
4	25	31	28
Mean	**23.25±1.25**	**31.75±0.957**	**27.5±0.577**

Dissolved oxygen

The dissolved oxygen (DO) concentration was measured in the range between 6.3-7 mg L^{-1} and mean value was 6.55± 0.310 during the experimental period. The maximum dissolved oxygen (DO) concentration was ranged between 8mg L-1 in this study period. The range oxygen recorded in this study was slightly varies but fell within the recommended values. This may be contributed by continues change of water and adequate aeration in all experimental.

The suitable dissolved oxygen for fresh water fish cultivation generally ranges from 4.5 - 8 mg L^{-1}(Bhatnagar, 2009). During the experiment period, there were slight fluctuations in dissolved oxygen in the tanks but they were under recommended range. This may be contributed by regular water change and adequate aeration with the help of blower.

Table 12: Dissolved oxygen concentration of nursery tanks during experimental period

DO Tank	DO of Week 1 (ppm)	DO of Week 2 (ppm)	DO of Week 3 (ppm)	Do of Week 4 (ppm)
1	6	5	8	6
2	8	6	5	5
3	8	6	6	6
4	8	6	6	6
5	6	8	8	7
6	8	7	7	8
7	8	6	6	6
8	6	5	6	5
9	6	6	6	8
10	6	8	6	8
Mean	**7±1.054**	**6.3±1.06**	**6.4±0.966**	**6.5±1.18**

pH range

The pH value of each nursery rearing tanks was mean7.9765±0.044 for study period. The highest pH value was 8.28 from nursery tank 9 in week 1 of the experiment period whereas lowest pH was recorded in week 3 with pH value of 7.54. The water pH values recorded for C. rubrofuscus breeding and fry rearing was 7.54-8.28. The average pH during the study was 7.9765±0.044 in nursery tanks and 7.9975± 0.320559 during breeding. According to The Fish Site, 2009 the suitable pH range for fish cultivation is from 6.5 to 9.0, a water pH level above and below this range will be stressful to the fish strain. Water PH of between 6.7- 8.5 is considered enough for fresh water fish cultivation (Adeniji 1987; Vivven *et al.* 1985). The water pH results showed no effect on fish fry mortality hence it was in a desirable range according to the data above

Table 13: pH range of nursery rearing tanks during experiment from week 1 to week 4 of raising period.

		pH		
Week Tank	**Week 1**	**Week 2**	**Week 3**	**Week 4**
1	7.89	7.65	7.66	8.13
2	8.24	7.71	7.54	8.21
3	8.16	7.96	7.78	7.97
4	8.11	8.11	7.57	7.67
5	7.96	7.91	8.16	8.16
6	7.74	8.21	8.26	8.21
7	7.81	7.91	8.11	7.88
8	7.86	8.11	8.6	7.73
9	8.28	7.93	7.87	7.68
10	8.17	7.66	8.23	8.27
Mean	**8.022±0.193**	**7.915±0.197**	**7.978±0.348**	**7.991±0.237**

NO_3 Concentration

No_3 concentration was measured using NO_3 test kit on a weekly basis. The NO_3 concentration was under control in the nursery tanks by frequent water changing and with the application of sponge filter. The highest NO_3 concentration was recorded 5 mg/L with lowest being 2.5 mg/l. the average NO_3 concentration level during experiment period was 3.75 ± 0.244 mg/ L. The ideal nitrate range for a proper growth and metabolism of fish is generally below 20 mg/L (Sharpe, 2021). The average nitrate concentration in the breeding tanks as well as nursery tanks during study period was 4.375±1.25 mg/l and 3.75 ± 0.244 mg/ L.

Table 14: Nitrate level in nursery tanks during experiment period

Week Tank	Week 1 NO_3 Concentration (mg/l)	Week 2 NO_3 Concentration (mg/l)	Week 3 NO_3 concentration (mg/l)	Week 4 NO_3 concentration (mg/l)
1	5	2.5	5	2.5
2	2.5	3	5	2.5
3	2.5	3	2.5	5
4	5	2.5	5	5
5	2.5	5	5	5
6	2.5	3	2.5	5
7	5	2.5	3	2.5
8	5	3	5	5
9	2.5	5	2.5	2.5
10	5	5	5	2.5
Mean	**3.75±1.32**	**3.45±1.09**	**4.05±1.24**	**3.75±1.32**

Breeding behavior and reproductive performance

The breeding season during the study was May which falls just before the peak rainy season. During this time, the ion concentration of the water decreases abruptly where most of the Asian strain of carp including koi starts to spawn (Towers, 2013). 10 sets of brooders were used during breeding where all of them showed breeding sign successfully during the following morning as suggested by Morris (2019). The fecundity of the fish bred with spawn pro with average weight 910±164.722 gm was found to be 63,900±6266.312. Freeman (1987) reported that the female C. carpio deposits eggs approximately 100,000 per kilogram of body weight. The slightly lower amount in egg number found in this study may probably be due to a smaller brood size.

The fecundity is also affected by many factors like age and size of the female, temperature and food availability (Nwokoye, Nwuba, & Eyo, 2007). The variation in fecundity within a common trail of similar-sized fish species could be attributed to hormone administration rate, breeding history, maturity stage, and other external environmental factors (Lager 1986; Schulz *et al.* 2007; Ataguba *et al.* 2009). Use of hormones may produce poor results if the brood fish are not well conditioned. Under such conditions a partial spawn or no spawning at all may occur, and others may not respond to hormone treatment even if they are in relatively good condition (Piper *et al.* 1982). The average fertilization rate of 68.6±7.1% was slightly higher than that to the experiment of Ghosh *et al.* (2012). Similarly, the latency period in the present study was 16 ±1.6 hours which is very long in comparison to 5-6 hrs obtained in the induced breeding by Sahoo (2020) and Ghosh (2012). According to FAO (1996) the latency period gets lower at higher and stable temperature.

The latency period of the study was longer due to randomly fluctuation in weather at night (21-24 ºC) times as the fishes were injected during evening hours. The average incubation period in the breeding chambers was 40±1.2hrs which was in accordance to Ghosh *et al.* (2012) that showed average incubation period of 40±2 hours at temperature 28-29 °C in summer. The variation in fertilization rate might be attributed due to varied egg and sperm quality, physiological difference of brood stocks, seasonal variation, as well as difference in hormone dosage (Gheyas *et al.* 2002; Haniffa and sridhar 2002; Nwokoye *et al.* 2007).

Environmental factors, water quality parameters (pH, oxygen concentration, hardness and temperature of water) and handling procedure of the brood fish also are determining criteria (Khan *Et al.* 2006). The fertility and fecundity of koi carp was relatively similar to other study due to favorable environmental conditions during stocking, relatively low spawning frequency and adequate food availability (Vazzoler, 1996). The study showed hatching rate of 75.5±5.9% which was comparatively lower than 95·33±2·08% hatching rate obtained by Yeasmin *et al.* (2016) and relatively close to 80% hatching rate obtained by Putri & Dewi (2019) in their study. The maximum hatching rate was 87.57% and minimum hatching rate was 70.52%. The poor hatching rate of spawned eggs could be linked to the exposure of fungus infection, where amount of dead eggs risked at being nutritional bases for fungal growth (Tucker and Robinson 1990). It was found that the fungi Saprolegnia spp. fungus commonly attack the C. carpio fertilized eggs during the incubation period. High egg densities, organic matter in fish hatcheries and due to presence of dead eggs, as they are considered the basic environment for microbial overgrowth hampered egg development and subsequently affect hatching rate (Yeasmin *et al.*, 2016)

Fry growth and survival

The sampling 250 eggs were reared in a nursery tank for 30 days including incubation time. The fries were fed with boiled egg yolk, artemia and moina as live food supplement along with crumbled pellets. The average mortality from ten different tanks after 28 days of nursery rearing at the end of the study was 11.76±1.37% with highest and lowest mortality rate being 13.49% and 9.22% respectively. Similarly, the average survivability rate from different nursery tanks was 88.9±1.59% which is comparable to that of Jha *et al.* (2006).

The survivability of the fries in our study was higher than that of Putri & Dewi (2019) study report. This might be due to additional live feedings given to the fries during their initial days of growth. The survivability rate of Jha *et al.* was comparatively little higher than my present study as the fries were reared in earthen ponds. According to Jha *et al.* (2006), introduction of live zooplankton into culture units' result in higher growth of koi carp larvae compared to manure-based systems. Earthen ponds appeared to be better alternative to concrete tanks for manure application through maintenance of better water quality due to their higher assimilatory capacity and greater abundance of plankton which resulted in better growth of cultured fish (Jha *et al.*, 2006).

Furthermore, any possibilities of risk were minimized by incubating 250 sample fries in their respective nursery tanks and any chances of predation from frogs, dragon fly larvae, birds were completely checked as the unit was completely under our supervision. The length gain by the fry during 28 days of nursing period was 17.935mm at the end of experiment which is similar to that of Haniffa *et al.* (2007) where length gain after 35 days was between 15-17 mm. Larval growth depends on the factors of feed quality and water quality. Besides that, a stocking density that is too high in the context of the water is also not good for the growth of the koi fish larvae. The food factor is very important; it requires a good amount and quality of food to increase the weight and length of the fish. Additional food has a positive effect on the growth rate of the fish (Dani *et al.*, 2005). The mean final weight of the fry in the nursery tank on 24 July, 2021 was 1.0845±0.0353 gm and and average weight gain after 28 days of nursery rearing was 0.9315 gm. The initial mean weight of the fry during hatching was 0.153±0.0014gm. The daily weight gain was 0.033gm per fish per day which is higher than that of Putri & Dewi. (2019), which might be contributed by optimum and healthy water quality parameters and live feed supplement (Khan *et al*. 2006).

CONCLUSION

The high fecundity, survivability, fast growth and simple breeding technique of the koi fish suggest they are suitable species for commercial culture in Nepal. Even though the experiment was carried out with limited facilities, the result was promising. The study result has the potential to be a guide line for further researches to improve artificial breeding of ornamental fish Koi in Nepal. It also aims to combine terms of optimization between environmental parameters and hormone application. During the observations, the koi length and weight growth continued to increase. The hatchery of the koi in Everest Aquaponics and fish farm, Sunsari, Nepal was good enough. This can be seen from the high FR, HR, SR and other growth parameters values. One of the influencing factors is the good quality of the water. Water quality parameters like average temperature of 26-28 ºC, dissolved oxygen above 5 mg/L, pH between 7-9 and Nitrate concentration below 5mg/L was found suitable for breeding and rearing of koi carp in Sunsari, Nepal. The breeding and rearing of koi carp to produce seed was found to be a profitable venture and even small investment in this can give good return in short span of time.

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