VIABILITY OF BREEDING *Colisa lalia* (F. Hamilton, 1822) AND LARVAL REARING ON DIFERENT FEEDING REGIMES

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RECOMMENDATIONS

This is to recommend that the thesis entitled **"Viability of breeding *Colisa lalia* (F. Hamilton, 1822) and larval rearing on different feeding regimes"** has been carried out by Niraj Khadka for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish and Fisheries. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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ABSTRACT

The present study describes the reproductive activities in different temperature of the dwarf gaurami, *Colisa lalia*, and investigates the survival rate and growth performance of larvae under various feeding regimes in captivity. Fishes were collected from wetland near Koshi River and domesticated in laboratory of Central Department of Zoology, Tribhuvan University. Fishes were fed mixed diets of pellet food, mosquito larvae and Artemia twice a day. To optimize water quality daily siphoning of waste and partial water was changed. Nine adult pairs were selected to observe its breeding behaviour and spawning performance on 3 different temperatures (22º C, 25º C and 28º C). Male built the bubble nest under floating object and female deposited her eggs on it. Among 3 temperature groups, only 25º C and 28º C produce viable eggs. Average number of eggs observed was 185, 215±10 and 195±18.03 for 22, 25 and 28º C temperature group. Male guards and fanning the eggs until it hatched. Eggs hatching started within 24-36 hours post fertilization. Larvae were fed on infusoria and rotifers for 20 days before feeding experiments. 45 similar sized (length 5.33±0.5mm and weight 3.53mg) larvae were used in feeding trial. During feeding experiments photoperiod 24L/0D was maintained. Using three different feeding experiments (live food, egg yolk and commercial pellet), survival and growth of larvae were studied for 30 days (up to 55days post hatching). Feeding experiment was conducted on 2 liter container. Total number of larvae used was 45, 5 in each jar. Highest survival rate (100%) was obtained from larvae fed on live food. Survival rate of larvae fed on egg yolk and commercial pellet were 66.67% and 46.67% were observed. Growth was also higher in live food fed larvae reaching final mean length, weight and SGR per day was 8.93±0.4 mm, 6.6±0.53mg and 5.57±0.33% respectively. That value for egg yolk was 6.43±0.72mm, 4.47±0.65mg and 3.84±0.71%. Similarly, on commercial pellet diet 6.13±0.78mm, 4.5±0.5 and 3.89±0.52 was observed. Result showed that the proper diet for successful larval rearing of this species was live food organisms. The results obtained in the present study reveals the efficiency of live food as better feed for larvae culture. Study concluded that, captive breeding can play a great role in the conservation of this species along with its habitat protection. This study is an initiation of breeding of indigenous ornamental fish and rearing of its larvae, which will form the basic platform for further research on this species.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
CP	Crude Protein
EE_2	Ethinyloestradiol
LED	Light Emitting Diode
Ln	Natural log
mg	Milligram
ml	Milliliter
mm	Millimeter
Ppt	Part per Thousand

1. INTRODUCTION

1.1 Brief History of Ornamental Fish

Ornamental fishes are aquatic organisms that are reared as pets and are generally kept for relaxation, entertainment and decoration as a hobby. It is an important commercial component for aquaculture and it is the one of the growing trade globally (Dey et al. 2014). Aquarium fish keeping began in 1805. The world's first public aquarium was maintained and opened in England in 1853 at the London zoo. Since then rearing and breeding of ornamental fish had increased (Dholakia 2009). The basic things to be considered in aquarium keeping are proper maintenance of water conditions like temperature, water flow, dissolved oxygen and provision of appropriate feed. Lighting, bottom substrate maintenance and selection of suitable fish are also important.

1.2 Biology of Dwarf Gourami (*Colisa lalia*)

Dwarf gaurami, *Colisa lalia* (Hamilton 1822), local name kotari/kotri is a small sized, brilliantly colored fish (Shrestha 2008). It is native to Nepal, Bangladesh, Pakistan and India. However it has now been widely distributed outside of its native range with feral populations found in Singapore, United States and Colombia. It prefers stagnant pond and pool where aquatic vegetations are densely found. It also inhabits in river and they are also reported from Koshi, Karnali, Narayani Rivers in Nepal.

C. lalia is an omnivorous fish feeding small invertebrates and algae. It lives at the surface of the water column. In poor oxygen condition, it can also breathe air through labyrinth organ, an accessory respiratory organ. Labyrinth organ is a densely folded suprabranchial structure made from vascularized expansion of the epibranchial bone of the first gill arch. If a labyrinth fish is restricted to access the air in poor oxygen conditions, it will drawn because the gills alone will not provide sufficient oxygen to the fish (Barman et al. 2013).

In nature they make nest using bubbles, plant twigs, leaves and other debris. Once the nest build is complete, male will start courtship display. It will flair its dorsal fin and swims around a female to draw her attention. If female is receptive, she will circle around male and go underneath the nest. After some breeding rituals, female starts laying eggs and male quickly fertilized releasing milts over them. The number of egg ranges from 300 to 600 (Hayakawa and Kobayashi 2012). Eggs are lighter than water so that they go to the bubble nest on the surface, those cannot go up are carried by male to the nest. Dwarf gaurami shows some level of parental care. Male fish guards the eggs from others fish until it hatched out and swim freely.

C. lalia is one of the most popular aquarium fish in world due to its sparkling colour and its hardy nature as they can tolerate low oxygen level and poor water quality (Shim 1987). It is also considered as good food fish due to their delicacy. Another peculiarity common to gouramis is the presence of long feeler like pelvic fins. It is used as tactile organ and even to greet other gouramis (Shrestha 2008). There is several variety of dwarf gaurami - Dwarf gaurami, Neon blue dwarf gaurami, Fire red dwarf gaurami etc. They are developed by selective breeding for specific trait. Ornamental fishes form an important commercial component of aquaculture and artificial breeding in captivity helps to conserve and maintain the stock at natural habitats (Mitra 2006).

1.2.1 Taxonomy (Hamilton 1822)

Order-Perciformes

Sub-order-Anabantoidei

Family-Belontiidae

Sub-family-Trichogasterinae

Genus-*Colisa*

Species-*lalia*

1.2.2 Synonyms

Tricopodus lalius (F. Hamilton, 1822)

Colisa lalia (F. Hamilton, 1822)

Colisa unicolor (G. Cuvier, 1831)

Polycanthus lalius (F. Hamilton, 1822)

Trichopsis lalius (F. Hamilton, 1822)

Trichogaster lalius (F. Hamilton, 1822)

1.2.3 Morphology

Body is egg shaped which is strongly compressed from lateral side. Mouth is small, protrusible. Male can reach a size of about 2.5 inches and females are little smaller. The principle color extending over the body of the male, excepting the fins and tail, is steel blue, with irregular orange zigzag bands running vertically. The fins and tail (except the pectoral fins) are orange, mottled with a great number of red spots of a pin-head. The edges of the fins are blue with bright red tips. During the mating and breeding seasons these already present bright colors are greatly intensified. The pale orange becomes redder and blue a most brilliant dark steel blue. Female fish is not nearly as colorful as the male, being a pale silvery-blue with a faint trace of orange-yellow vertical stripes from behind the gills.

Diagnostic Characters:

D 15-16/7-8; P 10; V 1; A 17-18/13-14; C 15; L_l 26-28; L.tr. $4^{1/2}$-$5^{1/2}$/10; TL=5 cm

1.2.4 Sex Determination

Sex determination can be done only after fish reached sexual maturity. Wild adult male dwarf gaurami has diagonal stripes of alternating blue and red colours; whereas females are silvery. Male reaches about 2.5 inch in total length and female is little smaller. Females with eggs have rounded belly where males have slender belly. Beside theses character sex can be differentiated by dorsal fin. Dorsal fin of male is pointed and long extended up to middle of caudal fin, while, that of, female is shorter and rounded.

1.2.5 Aquarium Condition for *C. lalia*

Colisa lalia naturally lived in ponds, ditches, streams where aquatic plants are plentiful. It needs warmer water at around 23-29^{0}C. It is small and peaceful fish and ideally suited to small to medium sized community tank. The tank should not contain any predatory or aggressive fish. It can be kept with tetras, small barbs, guppies etc. In natural conditions, it can be reared on omnivore diet such as animal and plant matter (algae). In natural habitat, fish and their fry feed on wide verity of food including micro-zooplanktons. Natural food supplies the necessary nutritional requirements of fish. This fish can reared in artificial diets also but proper balance of protein, lipid, carbohydrate, minerals, vitamins and other growth factors must be considered. Selecting proper food is important from nutritional and economical point of view. Successful aquaculture depends on a good knowledge on nutritional requirements of cultured fish.

1.3 OBJECTIVES

1.3.1 General objective

A. To evaluate the viability of breeding of *Colisa lalia* and larval rearing in different types of feed.

1.3.2 Specific objectives

a. To study the breeding behavior of *C. lalia.*

b. To investigate the viability of breeding of *C. lalia* at different temperature.

c. To compare the better feeds for larval rearing.

1.4 SIGNIFIANCE OF THE STUDY

In Nepal, very little study is done on ornamental fish. This study will give knowledge on breeding behavior of ornamental fishes like *C. lalia*. At the same time, it will help to understand the efficient larval rearing technique.

2. LITERATURE REVIEWS

2.1 Brief History of Ornamental Fish Breeding

Ornamental fish keeping is becoming popular hobby and is increasing day by day throughout the world. With the increasing demand of ornamental fishes, its farming is also growing. The USA, Europe and Japan are the largest markets for these fishes. But more than 65% of ornamental fish came from Asia (Ghosh et al. 2003). Before advance knowledge in breeding ornamental fish developed, there were only wild-caught fishes available in the market. Most of the wild-caught fresh-water fish came from Amazon River basin, the Congo River basin and the major rivers of Southeast Asia. These days, ornamental fishes are produced in farm which is easy and more sustainable method than wild-capture. Thailand, Singapore, Indonesia, Hong Kong and Malaysia are the major countries to breed and export ornamental fish (Watson et al. 1996).

Intensive breeding in Asian countries has without doubt produced socio-economic benefits for the local population, as well as creating new varieties of fish with different color variations or long fins. While such activities have helped stem the depletion of numerous species, reducing the probability of extinction, they have also produced notable biological and medical problems. As a result of intensive breeding and the abuse of genetic selection which tends to standardize size and color variations, the resistance of some of the most popular species (poeciliids, cichlids, Barbus spp.) had decreased with a subsequent increase in induced pathologies (Monticini 2010).

2.2 Breeding of Ornamental Fish in Captivity

The successful reproduction of neon tetra can be done at 22^0C water temperature; but temperature above 25^0C had negative effect on reproduction. Neon tetra spawners produced viable gametes for 5-6 spawning periods only. Fish can reproduce shortly after completion of spawning. Keeping the fish between spawning periods more than 20 days had a significant

deterioration of gametes quality at 25^0C (Kucharczyk et al. 2010). Breeding of Honey Gourami (*Colisa sota*) found to be successful under control environment without administration of any hormones. Fish laid 200-400 eggs at water temperature 28^0C in bubble nest built by male (Mitra et al. 2006). Reproductive behavior, the survival and growth of larvae of fork-tail blenny (*Meiacanthus atrodorsalis*) were studied under various feeding regimes (Moorhead and Zeng 2011). Courting behavior of spawner suggested that female initiated courtship with spawning commencing after a series of male displays and courtship encounters. Fish prefers cave like shelter for breeding which have smaller entrance. Fertilized eggs hatched after 181 hours at 28^0C water temperature. Male fish is responsible for caring the eggs. Dey et al. (2014) studied the breeding and development of ornamental fish *Devario aequipinnatus* in captivity without inducing hormones. The main stimulus for induction of spawning is the sudden cooling of aquarium by the artificial rain created with 4^0C cooler water and then gradual increase of temperature from 25^0C to 27^0C. Results suggested that temperature and rainfall played key role in breeding of this specie like its other congeners (*D. rerio and D. malabaricus*).

Methyl testosterone (17α-MT) can affect on the phenotype, bio-indices and gonads of male dwarf gaurami (*Colisa lalia*). Fishes fed with hormonal doses of 10 and 15 mg/kg feed showed elevated body color compared to 5 mg/kg feed. It shows that 10 mg/kg is optimal dose of 17α-MT on this species (Biswas et al. 2014). Bronstein (1982) studied the breeding, parental behavior and their interruption in *Betta splendens*. In this species, spawning occurs after brood pair introduced in tank for about 24 hours and male guards the eggs and fry thereafter. Author reported that presence of intruder male in vicinity decreases the reproductive efficiency of male. The rearing and breeding management of Pomacentridae, *Chrysiptera parasema* in captivity with proper diet and photoperiod is essential for survival of the *C. parasema* larvae. This study shows that, 100% mortality was obtained within 3 days and 7 days with photoperiod of 13L/11D and 16L/8D respectively on this coral reef fish (Olivotto et al. 2003). Santos et al. (2007) examined the gonadal transcriptome responses of exposure to oestrogen in breeding zebra fish (*Dania rario*). This study generates the mechanistic understanding of the disruptive effects of exposure to 17α-ethinyloestradiol (EE_2) on reproduction in zebra fish by anchoring the transcriptomic alterations induced with the physiological consequences. Results summarizes that EE_2 exposure compromised the

reproductive health of zebra fish by decreasing egg production and alterations in sperm quality.

The ovaprim can effect the breeding performances in fresh water angelfish (*Pterophyllum scalare*). The effect of different doses (0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25 ml/kg body weight) of ovaprim and accounts fecundity, response time and fertilization rate. The result shows that highest fecundity (665.66) and fertilization rate (95%) obtained from 0.35 ml/kg dose whereas minimum response time occurs in 0.4ml/kg dose (Chatterjee et al. 2013).

2.3 Larval Rearing on Various Diets

Fish fry rearing is considered to be bottle neck for every aquaculture practice. Fish larvae rearing experiments investigating nutritional factors or rearing protocols are carried out in various systems, from small beakers to very large commercial tanks, making it difficult to compare data across systems. It is shown that survival and growth of larvae was significantly increases fed with live feed. Density of live food and its size also play the vital role in survival of the larvae (Moorhead and Zeng 2011).

Rotifers density in the larval rearing tanks play minor role on survival and growth of Fork-tail Blenny (*Meiacanthus atrodorsalis*) when reared in captivity. However, switching from rotifers to Artemia nauplii at early stage affect negatively on survival rate of larvae because of their small mouth gape. Therefore selection of appropriate food for every stage of their life is crucial for their proper growth and survival (Moorhead and Zeng 2011). Olivotto et al. (2005) studied the breeding and larval rearing of Cleaner Goby (*Gobiosoma evelynae*). They reported the successful breeding of Cleaner Goby. They carried out trials where larvae were fed with different HUFAs enriched feed combinations of small to large zooplanktons. Higher survival rate of 50% was observed in larvae fed on smaller planktons compared to 10% fed on larger zooplanktons. Study concluded that appropriate size of food organisms has key role in survival of larvae of this species. Sundarbarathy et al. (2005) investigated the breeding of stone-sucker fish and rearing of larvae up to juvenile stage. They compare the growth rate of

larvae with different feed (formulated feed, live feed and dried swine liver). Result shows that higher growth of larvae observed on fed on dried swine liver up to 45 days. And over all highest growth attained was found on larvae fed on formulated feed.

Rotifers density have greater role on rearing of *Chrysiptera parasema* fish larvae. As lower density of rotifers leads to starvation and higher density create depletion of oxygen in water. It was found that, when *Brachionus plicatilis* was provided as the first food for larvae; optimum density was 20 rotifers per ml. And if rotifers density crosses the 28 per ml level, larvae could die due to depletion of oxygen. In this species, dissolved oxygen below 5ppm represents a critical point for larval survival (Olivotto et al. 2003). Patra and Ghosh (2015) studied the larval rearing of fresh water Angelfish (*Pterophyllum scalare*) on different diets. Authors observed survival rate and growth performance of fish larvae. Result shows that the highest survival rate (74.67) and growth rate were found on larvae fed on Artemia. Schlechtriem et al. (2004) compared the growth rate and survival rate of carp larvae fed on nematodes and Artemia. Larvae fed on Artemia shows 97% survival rate where larvae fed with nematodes have only 80% survival rate. Growth is also 5 times higher in Artemia fed larvae. It concluded that Artemia is better feed option for rearing carp larvae. Olivotto et al. (2006) investigated the importance of live prey enrichment during larval development of sunrise dottyback (*Pseudochromis flavivertex*). Authors tested 3 groups of feed to find the growth, larval survival and metamorphosis timing. Highest rate of survival was obtained from the live feed which was nutrient enriched over other group of live food which were partially enriched or not enriched.

2.4 Live Food Culture

Rotifers and Artemia were found to be excellent live food for culturing ornamental fish larvae. Rotifers were suitable for early stage of life (2-12 days) specially for smaller species because of smaller mouth gape of larvae and Artemia are suitable for later period (13-32 days) (Lim et al. 2003). Dhert et al. (2001) studied the culture technique and enrichment of rotifers. Author enriched rotifers by feeding them formulated culture diet rather than submerging food in oil emulsions. It shows better result on development of fish larvae. Study

also shows that rotifers could be cultured in higher density (up to 10 times) at re-circulating system. Rombout at el. (2003) studied the intensive rotifer culture system using nitrifying inoculums (ABIL). Study suggested that higher density of rotifers (5500 rotifers per ml) can be reared using nitrifying culture. It supports the higher growth at intensive culture of rotifers.

3. MATERIALS & METHODS

3.1 Collection and Transport of Fish

100 juvenile fishes were collected from wetlands on the banks of the Koshi River near the Koshi Barrage on September 2018 by using cast net with the help of local fishermen. After capturing the fishes, they were placed in the container of clean running water for 30 minutes. These fishes were starved for 24 hours to conditioning for transportation. Conditioning helps to decrease stress level during transport. Starvation also helps to reduce fouling of holding water and mortality during transportation (Mandal et al. 2010). Then the fish were packed in 10 polythene bags; 10 in each. Polythene bags were packed in such a way that one-third part was water and remaining two-third part was air.

3.2 Prophylaxis, Quarantine and Domestication

All the experiments were conducted in Laboratory of Central Department of Zoology. Upon arrival, they, inside the polythene bags, were acclimatized first for 30 minutes in tank water to reduce thermal shock. After unpacking them from polythene bags, they were kept in 2% potassium permanganate solution for 3 to 5 minutes as prophylactic measures. Then they were pre-conditioned to commercial feed for a week to ensure their good health. During it, fishes were closely observed for sign of illness and external parasites. At that time they were reluctant to take commercial pellet so they were offered mosquito larvae, brine shrimp and other live foods. Gradually fishes were habituated to take commercial pellet.

3.3 Rearing and Brood preparation

After this, fishes were stocked in 5 tanks; each tank containing 20 individuals. Rearing tanks did not have any substrate and tanks were 105 liter (600*450*380 mm) in size and all tanks

were maintained at 25^{o} C by using aquarium heater. Aeration was done with the help of aerators and biological filtration was done using sponge filters. Fishes were fed twice a day (11:00 A.M and 16:00 P.M) with commercial feed containing 25-35% CP and pellet size of 0.8-1.0mm. In addition to the commercial diet they were provided with live food also. To maintain water quality, waste and left over feed was siphoned out and 20% of total water was changed daily. A photoperiod of 10 hours of light and 14 hours of darkness was maintained by 20 watt LED tube light and digital automatic timer. After rearing for six months, fishes started showing secondary sexual characters on March. Color of male was vibrant and they started making territory. Then male and female were separated and reared in separate tanks at the density of 10 fish per tank. Fish were fed with live food (to trigger brood for spawning) and commercial pellets. Water parameters like Dissolved Oxygen, Ammonia and pH were recorded periodically.

3.4Behavioural Observation

Observation of reproductive behaviour of *C. lalia* was done in experimental tank which was stocked with one male and one female. Direct observation method was applied daily from 12 pm to 2 pm. Nesting behaviour, territorial behaviour, mating ritual and parental care were noted.

3.5 Spawning set up

Dwarf gourami can be bred in small tank of one gallon container (Cole et al. 1999). Therefore, spawning tanks of 450 x 300 x 380 mm were used for the experiment. There were 3 trials done to determine better condition for breeding/spawning. Each trial has 3 replications. Trials were temperature dependent. Trial 1 was set at 22^0C (named A), Trial 2 was set at 25^0C (named B) and Trial 3 was set at 28^0C (named C). Aquatic plants were kept in spawning tanks to mimic their natural spawning condition. Plants like *Rotala indica, Hydrilla verticillata and Pistia stratiotes* were kept in aquarium. The reduction of light by dense plants had a positive effect on nest building of fish (Degani 1989). Water level of tank

was kept at 18cm and photoperiod of 12 hours of light and 12 hours of darkness was maintained for spawning. Filters were not kept in tanks as they could destroy bubble nest. 45mm x 45mm Styrofoam was placed on the corner of tanks under which fishes made their nest. Feeding was similar to the brood preparation period. Spawning activities were closely monitored and recorded. Female fishes were immediately removed from tank after egg laid and only male fishes were allowed to take care of the eggs till they were hatched to free swimming stage. Eggs and fries were photographed and counted.

3.6 Rearing of Fry

Fry of all tanks were counted and congregated in one tank after free swimming stage and they were reared for 20 days. They are fed with infusoria and rotifers. The average growth rate was recorded in every 5 days measuring 20 randomly selected fries. All experimental groups were subjected to an extended photoperiod of 24L/0D to enabling larvae to feed longer period of times (Olivotto et al. 2005). There were 3 trials to determine better feeding regimes. Every trial had 3 replications. Experiments were conducted on 2 liter plastic jar and each jar containing 5 fries. Trial 1 was given live food, Trial 2 was given boiled chicken egg yolk and Trial 3 was given commercial fry food. Mortality of fry was examined daily and noted. Growth was determined at the end of experiment after 30 days by measuring fries size to nearest 0.5mm.

3.7 Estimation of Survival Rate

The survival rate of *C. lalia* larvae for each treatment and replication was recorded at the end of experiment.

The survival rate was calculated thus,

$$Survival\ Rate(\%) = \frac{No.of\ actual\ fish\ survived}{No.of\ actual\ fish\ stocked} \times 100$$

3.8 Growth Estimation of fish larvae

Initial and final length and weight of the fish larvae (n=45) was used to determine growth performance using following formulas:

$$\text{Length gain (\%)} = \frac{\text{Average final lenght} - \text{Average intial length}}{\text{Average initial length}} \times 100$$

$$\text{SGR } (\%d^{-1}) = \frac{Ln\ final\ weight - Ln\ initial\ weight}{Number\ of\ experimental\ days} \times 100$$

3.9 Live Food Culture

Various live food species (Mosquito larvae, Daphnia, Rotifers, Artemia and Infusoria) were cultured in laboratory to feed the adult *Colisa lalia* and its larvae following standard protocols (Dholakia 2009).

3.9.1 Mosquito larvae

Mosquito eggs were collected from bucket which was filled with aquarium water and placed on sunny area for 48 hours. Collected eggs were then placed in 40 liter tank inside the laboratory to develop into larvae. Larvae were collected daily using fine mesh net and fed to the fish. Mosquito larvae culture was started onset of warmer month.

3.9.2 Artemia

Commercial Artemia/Brine Shrimp decapsulated cyst was bought from aquarium shop. To hatch it, 2 liter plastic jar was used. Process of hatching of Artemia cysts are as follows:

Step 1: Plastic jar was filled with 30 ppt salt water.

Step 2: Two grams of cysts was poured in jar and mild aeration was provided by air pump.

Step 3: After 24 hours, light and aeration was turned off. The newly hatched Artemia nauplii settled to the bottom and shell comes to the surface. They were collected from bottom by pipette and separated by plankton net.

Step 4: Nauplii were transferred to 100 liter tank with same salinity and temperature to raise them. They were fed with spirulina and dry yeast powder.

Culturing of Artemia was done during entire experimental period. Newly hatched nauplii were fed to larvae and fry. Similarly, adult Artemia was used to feed adult fish.

3.9.3 Infusoria

They are single celled animals and are suitable for larval fish. Culturing of infusoria was started 1 week before spawning of fish. 40 liter tank was used to culture infusoria. A Tank was filled half with aged aquarium water and added rotten vegetable and banana peels. Tank was covered with net to avoid insects. After 4 days, culture water was smelly and appeared cloudy. Then water turned clear again after 6 days which indicates development of infusoria. Culture water was examined qualitatively and quantitatively before feeding the fish.

3.9.4 Daphnia

35 daphnias were collected from aquarium shop and examined under microscope. Daphnia was first kept in 30 ml glass test tube containing 20 ml of water. Each test tube was stocked with 5 specimens. After 24 hours their number was counted and they were transferred to 100 ml beaker. Their number was counted again and transferred to 40 liter aquarium for further rearing after next 24 hours. Culture tank was maintained at 22^0C temperature. Daphnia was fed with *Spirulina* and dry yeast powder. Its density was estimated daily by counting number of daphnia in 10 ml of culture water sample.

3.9.5 Rotifers

Disease free stock of rotifers (*Brachionus* sp) was collected from fish free pond of Kirtipur by using plankton net. Rotifers were separated from other planktons using microscope and dropper. Culture was provided with dry yeast and *Spirulina* powder.

3.10 Physico-chemical Parameters

The temperature, dissolved oxygen, pH and ammonia of the aquarium water were monitored throughout the experiments.

- Temperature was monitored by aquarium thermometer.
- Dissolved oxygen was measured using Winkler method.
- PH was monitored by Hanna ph meter.
- Ammonia was monitored by using API Freshwater Master Test kit.

4. RESULTS

4.1 Reproductive behavior

4.1.1 Sexual Dimorphism

After rearing for six months, secondary sexual characters were clearly noted (on the month of March). Mature female can be easily recognized due to the presence of rounded, swollen belly and round dorsal fin (Photo 1a). Mature male was slim, vibrant colored and pointed dorsal fin (Photo1b).

Photo1. (a) Mature adult female *C. lalia* (b) mature adult male *C. lalia*

4.1.2 Territorial behavior of male

Male showed unique character of making territory. After introduction of mature male and female fish in spawning tank, fishes began to display their breeding activities. During which, male fish showed most intensified color and flair its dorsal fin to impress female. Male stimulated female by touching her ventral side by its mouth. They mate several times at the interval of 30 seconds to one minute (Photos 2 and 3).

Photo 2. Male making territory under Submerged plants

Photo 3. Spawning pair and their nest

4.1.3 Nest building by male

Male started cleaning nesting sites and made bubble nest under the Styrofoam and aquatic plants. It was the first indication of beginning of the breeding. After completion of nest, male started to chase female and push under the nest (Photo 4).

Photo 4. Bubble nests under Styrofoam and under aquatic plants

4.1.4 Bubble nests

The time taken to build bubble nests were depended upon temperature. Time taken was recorded higher at low temperature and vice versa. Similarly, the size of nest developed was also found depended upon the temperature, however, the time taken and size of nests were similar in both 25^0C and 28^0C (Table 1).

Table 1. Bubble nests.

S.N	Trial	Time taken to make bubble nest (in hours)	Size of bubble nest (In cm^2)
1	T_A (22^0C)	72	143.88
2	T_B (25^0C)	32±13.86	38.03±8.11
3	T_C (28^0C)	24	47.85±11.98

4.1.5 Spawning behavior, eggs laying and male caring eggs

The weight of female at the time of breeding was measured 1.27, 1.36, 1.19, 1.26, 1.33, 1.21, 1.28, 1.31 and 1.25 gm. Male wrapped around female to exert pressure on later body to expel eggs out. The eggs were buoyant, came to the water surface and enter bubble nest. Scattered eggs were collected by male to bring to nest. After spawning, females were removed from the tank and males were left to take care of those eggs. Average number of eggs in trial A, trial B and trial C was recorded as 185, 215±10 and 195±18. The average number of free swimming larvae found 0, 165±5 and 150±10 in trial A, trial B and trial C (Table 2).

Table 2. Reproductive activities of *Colisa lalia.*

S.N	Trial	Number of eggs	Number of free swimming fry
1	T_A (22^0C)	185	0
2	T_B (25^0C)	215±10	165±5
3	T_C (28^0C)	195±18.03	150±10

4.2 Larval Development and rearing

4.2.1 Development of Fish larvae

The eggs were small and transparent. Hatching started within 24-36 hours after fertilization. Newly hatched larvae remained attached to the nest or walls of the spawning tanks. They

contained yolk sac in their abdomen and did not feed. The hatchlings became free swimmer on 72 hrs to 96 hrs. The first food (infusoria) was offered to the free swimmer after the yolk sac was almost reabsorbed. The newly hatched larvae measured approximately 1.9 mm total length and have transparent body (Photo5).

Photo 5. Newly hatched larvae

4.2.2 Larval Rearing

After 25 days of rearing, larval forms reached average length of 5.33±0.5 mm and weight of 3.53. After 30 days of feeding trial, final mean length of larvae recorded were 8.93±0.4, 6.43±0.72 and 6.13±0.78mm for live food (LF), egg yolk (EY) and commercial pellet (CP) respectively. Similarly, the mean final weight of the larvae attained were 6.6±0.53, 4.47±0.65 and 4.5±0.5 mg for LF, EY and CP respectively.

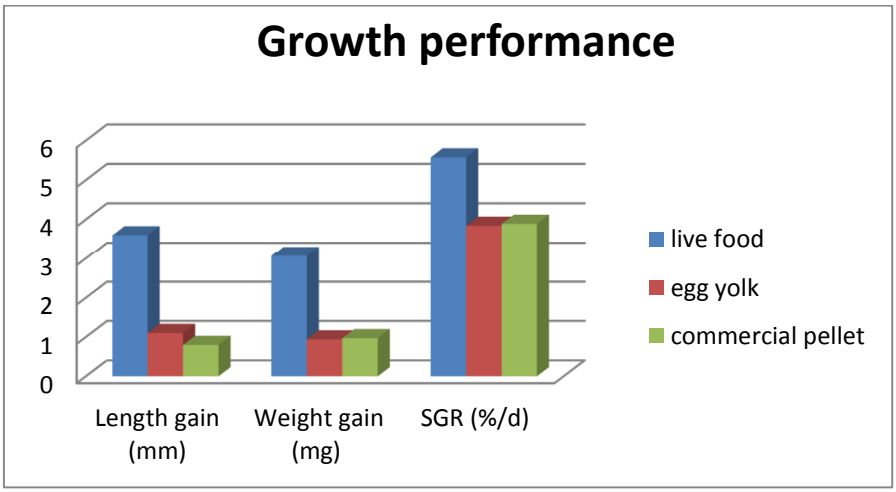

Fig 1(a). Comparative growth performance of larvae at feeding different diets.

The highest length gain (3.6±0.4 mm) and length gain percentage (67.5±7.58%) were observed in larvae fed on live food. The length gain (1.1±0.72 mm) and length gain percentage (20.63±13.41%) was second highest in egg yolk. The length gain (0.8±0.78 mm) and length gain percentage (15.01±14.63%) was lowest in commercial pellet feed (Table 3; Fig. 1a and 1b).

Table 3. Growth performance of larvae in various feeds.

Treatment	Replication	Length gain (mm)	Length gain (%)	Weight gain (mg)	Weight gain (%)	Survival (%)	SGR (% day^{-1})
Live food	X1	4.07	76.36	3.67	103.97	100	5.94
	X2	3.37	63.23	2.87	81.3	100	5.46
	X3	3.37	63.23	2.67	75.63	100	5.32
	Mean	3.60±0.4	67.54± 7.58	3.07± 0.53	86.97± 15	100	5.57± 0.33
Egg yolk	Y1	0.3	5.63	0.22	6.23	80	3.04
	Y2	1.34	25.14	1.13	32.01	60	4.08
	Y3	1.67	31.33	1.47	41.64	60	4.4
	Mean	1.10± 0.72	20.63± 13.41	0.94± 0.65	26.63± 18.31	66.67± 11.55	3.84± 0.71
Commercial Pellet	Z1	1.67	31.33	1.47	41.64	20	4.4
	Z2	0.17	3.19	0.47	13.31	40	3.36
	Z3	0.55	10.32	0.97	27.48	80	3.92
	Mean	0.80± 0.78	15.01± 14.63	0.97± 0.5	27.48± 14.17	46.67± 30.55	3.89± 0.52

The highest weight gain (3.07±0.53mg) and weight gain percentage (86.97±15.0%) were observed in larvae fed on live food. The weight gain (0.97±0.5mg) and weight gain percentage (27.48±14.17%) was second highest in commercial pellet feed. The weight gain (0.94±0.65mg) and weight gain percentage (26.63±18.31%) was lowest in egg yolk (Table 3; Fig. 1a and 1b).

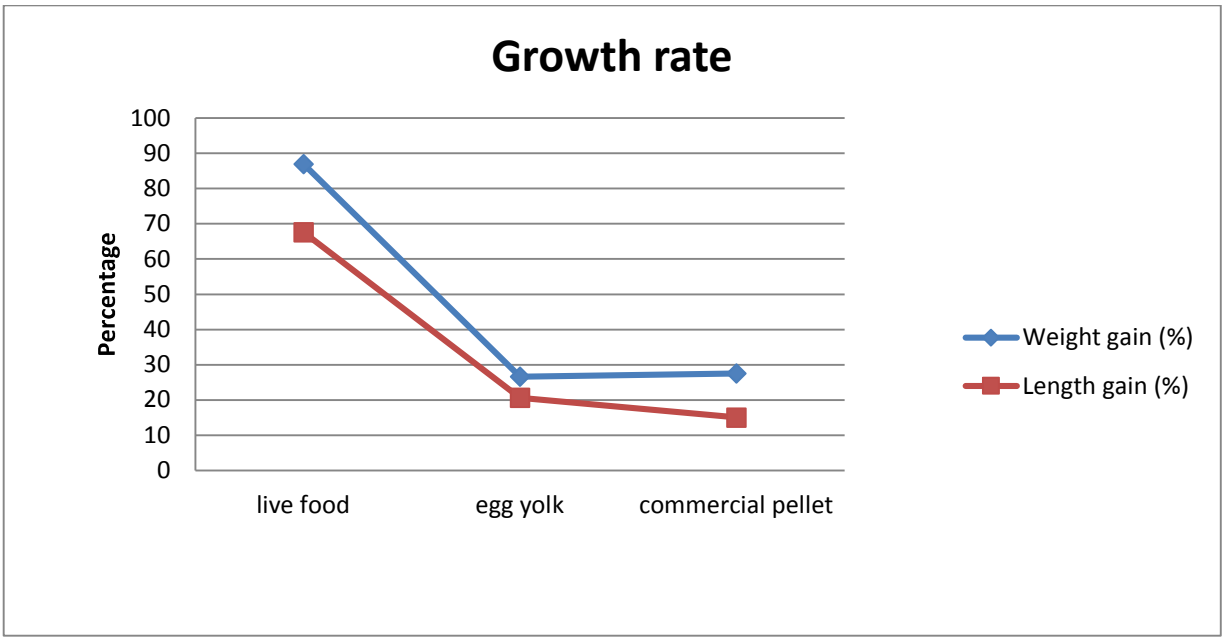

Fig. 1(b). Comparative growth rate at different diets

Highest survival rate (100%) was observed in larvae fed on live food followed by egg yolk (66.67±18.31) and commercial pellet feed (46.67±30.55) (Fig. 2b). Specific growth rate was recorded 5.57±0.33, 3.84±0.71 and 3.89±0.52 % per day for LF, EY and CP respectively (Table 3; Fig.1c).

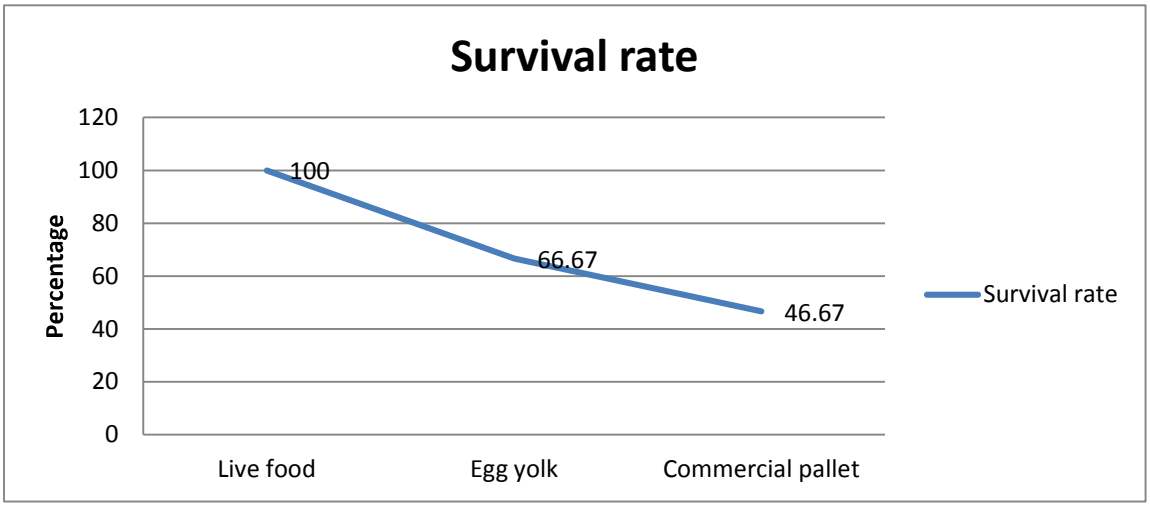

Fig.1(c). Survival rate of larvae after feeding trial of 30 days

4.3 Water quality

4.3.1 Water quality parameters in brood rearing tanks

Dissolved oxygen was recorded varying from 6.0 to 3.2 mg/l. It was found highest in Tank 1 and lowest in 3. However, DO were more or less similar in Tanks 2, 4 and 5. Ammonia was recorded highest in Tank 4 and lowest in Tank 1. But, Tanks 2, 3 and 5 showed similar value. The pH values were found more or less similar in all experimental tanks (Table 4). The temperature was maintained at 25^0C in all tanks as this temperature found optimum for *Colisa lalia.*

Table 4. Water quality parameters in brood rearing tanks (Temp at 25^0C).

Parameters	**Tank 1**	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
Dissolved oxygen (mg/l)	6.0	4.9	3.2	5.3	4.1
Ammonia (mg/l)	0.7	0.9	0.9	1.3	1.0
pH	8.3	7.9	8.6	8.2	8.0

4.3.2 Water quality parameters in spawning tanks

The water temperatures were recorded varying and gradually increasing in trials A, B and C. At the same time, dissolved oxygen was noted slightly decreasing from Trial A to Trial C. The data showed the inverse relationship between water temperature and dissolved oxygen. Ammonia was recorded 0.15 mg/l only in Trial A. pH was slightly lower in Trial A and similar in Trials B and C (Table 5).

Table 5. Water quality parameters in spawning tanks.

Parameters	**T_A**	**T_B**	**T_C**
Temperature (^{0}C)	22	25	28
Dissolved oxygen (mg/l)	6.3	6.2	5.8
Ammonia (mg/l)	0.15	0	0
pH	7.2	7.8	7.7

4.3.3 Water quality parameters in larval rearing tanks

The temperature was maintained at 25^0C in all larval rearing trials as this temperature found optimum for *Colisa lalia.* Dissolved oxygen was recorded lowest (6.5 mg/l) in Trial X and highest (7.2 mg/l) in Trial Y. Ammonia was not recorded in all experimental trials. Lowest pH (7.3) was found in Trial Y and more or less similar in Trial X (7.6) and Trial Z (7.7) (Table 6).

Table 6. Water quality parameters in larval rearing tanks

Parameters	**Trial X**	**Trial Y**	**Trial Z**
Dissolved Oxygen	6.5	7.2	6.9
Ammonia	0	0	0
pH	7.6	7.3	7.7

5. DISCUSSIONS

5.1 Reproductive Behaviour

The present study demonstrated the successful breeding of *Colisa lalia* in captive conditions without the use of any hormones. At the same time, successful larval rearing was carried up to 55 days with the administration of different diets. *C. lalia* appeared to adopt well in relatively small culture tanks. Otherwise, it is found difficult to breed wild caught fish in captive conditions due to the changes in environments, behaviour and physiology during domestication process (Krejszeff et al. 2009). Most wild caught ornamental fishes were reported to spawn in captive condition only after the manipulation of tank environments (Krejszeff et al. 2009). In the present study, the main stimuli for the induction of spawning were warm water temperature (25^0C), long photoperiod (12L/12D) and the presence of aquatic vegetations in spawning tank. *Colisa* sp. typically inhabits stagnant ponds, pools and slow-moving streams (Shrestha 2008). Therefore, maintenance of gentle air flow by sponge filter in rearing tank and lack of filter in spawning tank might have created the conditions like natural habitat.

It was found that, after providing suitable husbandry and appropriate environmental variables, fishes started to show secondary sexual characters i.e. intensive colouration, body shape etc. Brooder showed indication of imminent spawning by displaying territorial behaviour, cleaning of nesting sites, plumped belly of female. After introduction of breeding pair in spawning tank, male fish made bubble nest and stimulate female through touching her ventral side by his mouth and was followed by courtship and then mating. This behaviour is almost similar with the results of Hayakawa and Kobayashi (2009 and 2012). *Betta splendens* also showed the similar behaviour where male mouthing its mate's genital papillae to stimulate for spawning (Bronstein 1982). This breeding behaviour is also similar to other species of its genus like *C. fasciatus, C. sota* (Mitra et al. 2006; Barman et al. 2013 and Islam et al. 2016). According to Hayakawa and Kobayashi (2012), female eats their own eggs after fertilization; therefore, females had to be removed from spawning tank immediately after fertilization. Otherwise male could become aggressive and kill the female to save the eggs. Similar action had to be performed while breeding *Betta splendens* to protect female from aggressive male (Bronstein 1982).

5.2 Breeding Performance

Among three temperature dependent spawning trials, the first spawning event occurred in trials where fish were kept in 28^0C. At 28^0C, mean time to make bubble nest was 24 hours and longest time (72h) taken was found on 22^0C trials. Out of three in these trials, only one best bubble nest was observed. Since *C. lalia* are tropical fish and prefers warmer water to live and reproduce (Shim et al. 1987). Although the size of bubble nest was found largest in 22^0C tank, no viable eggs were observed and it contained least number of eggs. The reason for three and half time larger nest size was not clear, but, this might be due to the reconstruction of nest spreading in larger area after it was made. Cole et al. 1999 studied the reproduction of *Trichogaster trichopterus*, which had similar habitat like dwarf Gaurami, reported that nest building and egg deposition occurred in a temperature range of 23-29^0C, and there was no spawning in temperature of 20^0C. In this study largest number of eggs (215±10) was found in 25^0C temperature followed by 28^0C and 22^0C. It was difficult to distinguish the eggs from nest as eggs were transparent in colour and similar size as the bubble nest. Due to this reason number of eggs was observed low than the average number of eggs (300-600) produced by this species (Hayakawa and Kobayashi 2009). This experiment showed egg kept on temperature of 25^0C or higher had more than 76% hatched into larvae. Cingi et al. (2010) studied the effect of temperature on fertilization rate of *Coregonus lavaretus* and suggested that if the fish is out of suitable range of temperature, it adversely affect the rate of fertilization. Based on the results of the present study, breeding performance of *Colisa lalia* could relate to the temperature dependent. And it was found that 25-28^0C is preferable temperature for viable spawning.

5.3 Larval Rearing

In the present study, higher survival rate (100%) and better $SGRd^{-1}$ (5.57±0.33) was observed in larvae fed using live food organisms (Artemia nauplii, rotifers and daphnia); on the other hand, lowest survival rate (46.67±30.55) was observed in larvae fed on commercial pellet food. In nature, fish larvae fed on a wide variety of natural organisms like rotifers, infusoria,

protozoan, copepods, algae etc (Olivotto et al. 2005). Moorhead and Zeng (2011) reported that survival and growth of fish larvae strictly depended on the appropriate feeding and environmental conditions. It is commonly accepted that larvae need higher protein intake for their development. In experiments, average protein content of live food, egg yolk and commercial pellet were 56%, 25.6% and 48% respectively. The protein content of live food was higher than others. It suggested that protein is one of the many factors that control the growth. Fish larvae attained highest mean length of 8.93mm from 5.37mm in 30 days experiment. It was found very slow growth compared to its normal growth. The reason behind the slow growth could be the lack of enough food in culture tank. It was estimated that each larvae needs to consume roughly 500-100 micro-planktons each day (Wilkerson 1998).

5.4 Water quality

Water quality is an important integral part of any aquaculture system. It plays a major role in fish health and any deterioration in water quality causes stress to fish and brings about diseases (Arulampalam et al. 1998). Each water quality factor interacts with and influences the other parameters, sometimes in complex ways (Joseph et al. 1993). A good water condition is a necessity for the survival and growth of fish since the entire life process of the fish wholly dependent on the quality of its environment (Bolorunduro and Abdullah, 1996). In the present study, water temperature, pH, Dissolved Oxygen and ammonia were analyzed.

5.4.1 Water Temperature

Water temperature was maintained at 22^0C, 25^0C and 28^0C in spawning tank. From the study, 25^0C was found optimum with better remaining water quality parameters (Table 5). Since *C. lalia* are tropical fish and prefers warmer water to live and reproduce (Shim et al. 1987). Water temperature is controlling factor for all aquatic life. All biological and chemical processes in an aquaculture operation are influenced by temperature. At

temperatures above or below optimum, fish growth is reduced and mortalities may occur at extreme temperatures (Joseph et al. 1993). Boyd (1982) reported that the range of water temperature from 26.0 to
32.0^{0}C is suitable for warm water fish culture. Research has shown that a temperature range between 25 and 32^{0}C is ideal for tropical fish culture (Bolorunduro and Abdullah, 1996).

5.4.2 Dissolved Oxygen

Dissolved oxygen was recorded varying from 3.2 - 6.0 mg/l. It was found lowest in Tank 3 and highest in 1 at brood tanks (Table 4). However, DO were more or less similar in Tanks 2, 4 and 5. At the same time, dissolved oxygen was noted slightly decreasing from Trial A to Trial C. The data showed the inverse relationship between water temperature and dissolved oxygen at spawning tanks (Table 5). In larval rearing tanks, DO was recorded varying from 6.5 – 7.2 mg/l (Table 6). Dissolved oxygen is an important parameter in water quality assessment and reflects the physical and biological processes prevailing in the water. DO concentration of 5.0 mg/l throughout the year in the reservoir is productive for fish culture (Tarzwell 1957, Banerjee 1967). DO concentration in water is mainly dependent upon temperature, dissolved salts, velocity of wind, pollution load, photosynthetic activity and respiration rate. Oxygen levels never fell below 4.0 mg/l, which is considered to be the critical level for tropical fish rearing (Mallasen et al. 2012).

5.4.3 Ammonia and pH

Ammonia was recorded highest in Tank 4 and lowest in Tank 1. But, Tanks 2, 3 and 5 showed similar value. The pH values were found more or less similar in all experimental brood tanks (Table 4). Ammonia was recorded 0.15 mg/l only in Trial A. pH was slightly lower in Trial A and similar in Trials B and C in spawning tanks (Table 5). Ammonia was not recorded in all experimental trials. Lowest pH (7.3) was found in Trial Y and more or less similar in Trial X (7.6) and Trial Z (7.7) in larval rearing tanks (Table 6). Ammonia-N

(NH_3-N) is the principal nitrogenous waste produced by aquatic animals, via metabolism and is excreted across the gills (Cao et al. 2007). Ammonia strongly influences the dynamics of the dissolved oxygen in water, since 4.6 mg of oxygen is needed to oxidize 1.0 mg of ammonia. Ammonia levels between 3.0 and 4.0 mg/l may be toxic for tropical fish (Boyd 2001). Safe concentration of ammonia for freshwater fish is less than 0.05 mg/l (Lawson 1995). Ammonia concentration of 0.02 mg/l is required for optimum health of warm water fish culture (EPA 1973 and Jhingran 1988). Hydrogen ion concentration plays a significant role in the productivity. Normally pH range from 6.4 to 8.3 is favourable for fish growth (Robert et al. 1940). Hepher and Pruginin (1981) reported that this value ranging from 6.5 to 9.0 is good for fish culture. The water quality was recorded suitable for spawning, larval rearing and brood fishes in present study.

6. CONCLUSIONS

This study documents the breeding activities of ornamental fish Colisa lalia in captivity as well as investigated the growth performance of its larvae under different diets. Present study revealed the suitable range of temperature for breeding this species which was found 25-28C. And this species showed the some level of parental care (making territory and bubble nest) by male fish. The results obtained in the present study unfold the efficiency of live food as better feed for larvae culture. As higher survival rates and better growth were observed in larvae fed on live food than other groups. Thus, it gives clear massage about the importance of live feed culture in successful larval rearing of this species. Further studies on life history from natural conditions are needed.

7. RECOMMENDATION

Despite the market and commercial prospects of Gourami (*Colisa lalia*), its captive breeding in our country is not explored. Although majority of commercial fresh water aquarium fishes are breed in captive conditions. However, significant numbers of species are still captured from the wild. Therefore, captive breeding can play a great role in the conservation and commercialization of this species. This study is an initiation of breeding of indigenous ornamental fish and rearing of its larvae, which might be basic platform for further research on this species. This study was only confined to 22º C-28º C temperature. As *Colisa lalia* is warm water fish, further studies should be conducted at higher temperature as well as life history from natural conditions.

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PHOTO PLATES

PLATE 1

Experimental Male Brood Fish

PLATE 2

Experimental Female Brood Fish

PLATE 3

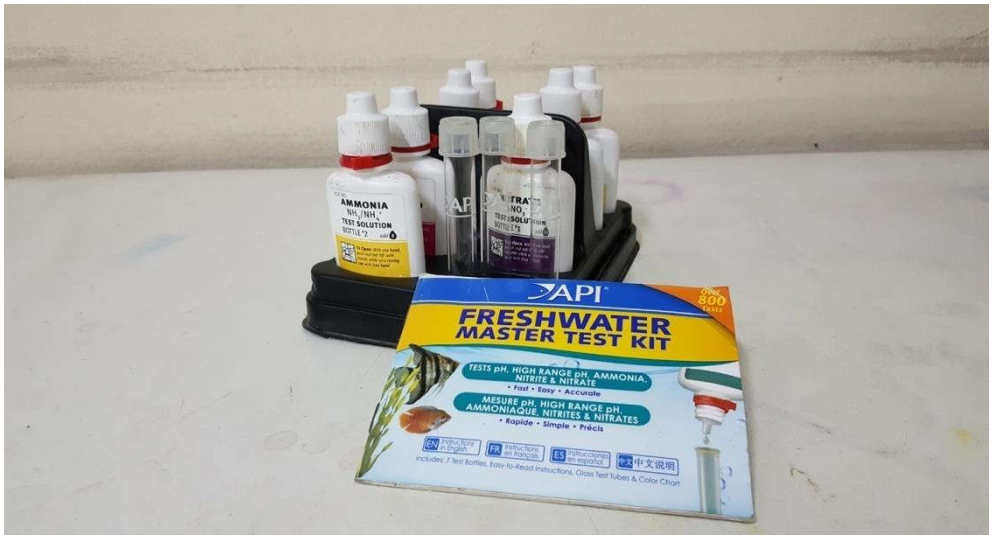
Breeding activities

Male fish under floating plant

Spawning pair and their nest

PLATE 4

Water Parameter Test



Water test kit

Testing parameter of tank water

PLATE 5

Breeding Tank Setup

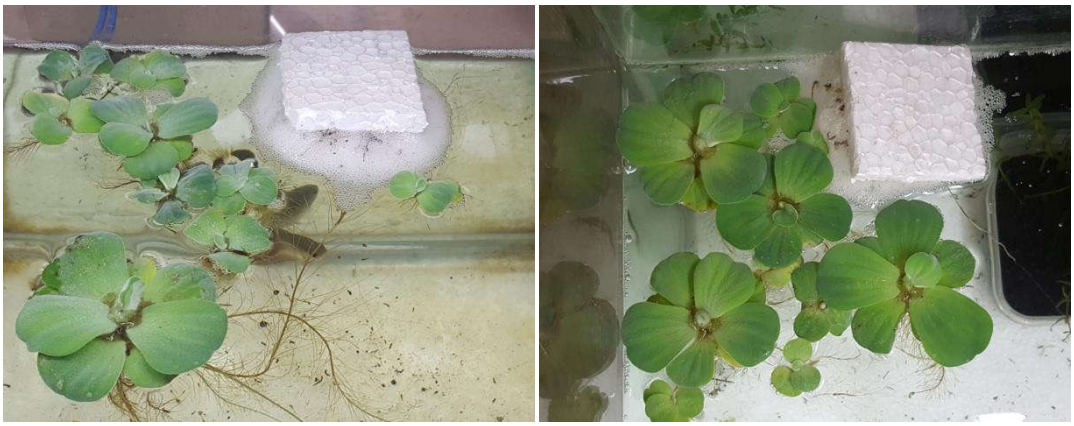
Experimental tank set up for spawning

Close up view of spawning tank

PLATE 6

Bubble nest and Environment of spawning tank

Submerged aquatic plants in spawning tank

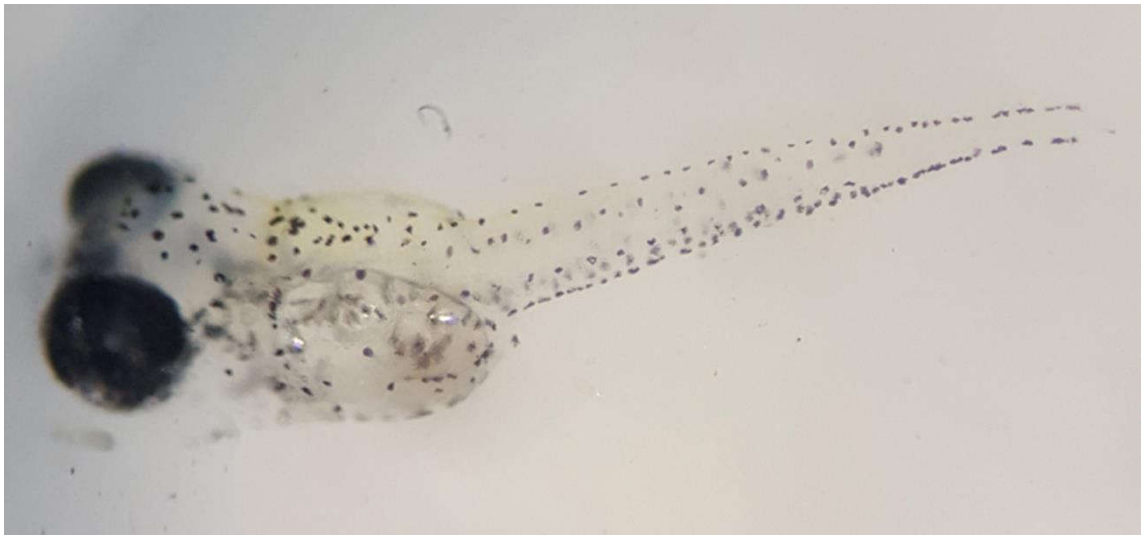


Bubble nest under Styrofoam

Bubble nest under floating plants

PLATE 7

Fish Larvae at different stages



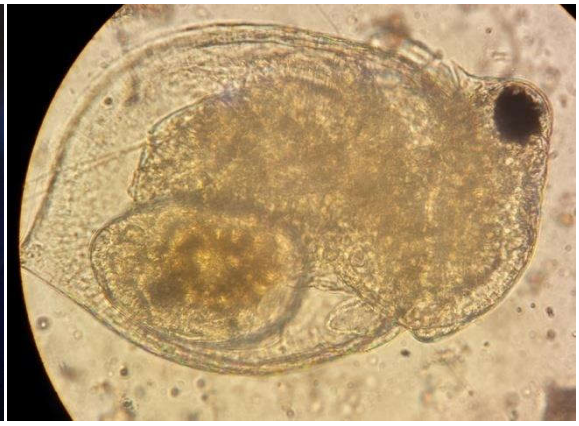
Larvae under microscope

Different size of larvae

PLATE 8

Some of live food organisms

Adult Artemia

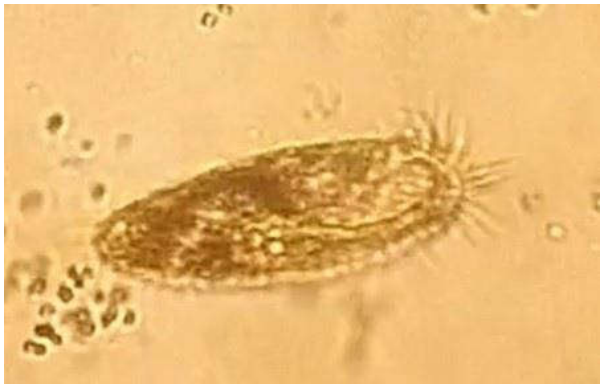


Daphnia

Artemia nauplii

Mosquito larvae

Rotifer



Paramecium