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Endogenous feeding period of rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the raceway ponds of Kathmandu, Nepal

Rakesh Prasad Bhagat and Sudip Barat

Abstract
Endogenous feeding period of 22 months old rainbow trout, *Oncorhynchus mykiss* (Walbaum), was recorded in the farmers' raceway ponds. The conversion and change of zygotes to alevins and from alevins to free swimming fries during developmental process and growth were less releasing small-sized alevins and free swimming fries because of the small-sized eggs and small age and size of the broods. Total incubation period was 30 days in water temperature of 9.1-13.1 ˚C. Hence, incubation period was 25 days in water temperature of 10.89 ± 0.21 ˚C day^{-1} and cumulative water temperature (sum total of water temperature of 25 days) of 272 ˚C with hatching of alevins (survivability 41.82 ± 3.16%), each 0.0365 ± 0.0016 g and 1.46 ± 0.086 cm with 0.0136 ±0.0012 g yolk-sac. Therefore, endogenous feeding period was 5 days in water temperature of 9.1 ˚C liberating free swimming fries (survivability 76.92 ± 4.91%), each 0.025 ± 0.0007 g and 1.65 ± 0.083 cm ready for the exogenous feeding period.

Keywords: Conversion and change, incubation period, alevins, endogenous feeding period, free swimming fries

1. Introduction
Rainbow trout, *Oncorhynchus mykiss* (Walbaum) 1792 (Anonymous, 2016) [4] mostly require crystal-clear cold water with high dissolved oxygen (DO) and suitable power of hydrogen ion concentration (pH) from water resource (WR) either spring-, glacier- or lake-fed rivulet and stream at high altitude (ALT) for its artificial breeding and commercial growth. The success of rainbow trout cultivation has been subsisted on physico-chemical parameters, breeding performance, and artificial feed. So, if physico-chemical parameters are suitable, breeding performance is successful and formulated artificial feed is well fed, then rainbow trout culture becomes feasible. Induced method of breeding is the successful method of breeding of any fish species including rainbow trout; therefore, it is artificially bred for obtaining fry and fingerlings for culture practices. The breeding, in Nepal, occurs from the first week of November to the beginning of December; however, it is bred once a year from November to February in both the Government Fish Farm at Godawari and Trishuli (Basnet *et al*., 2008) [5] and other private trout farms in the surroundings.

Artificial breeding performance of rainbow trout primarily depends on water quality parameters of the raceway ponds in which all types of broods are cultivated; secondarily on surveillance of disease occurrence in the broods, if any; highly on selection, management, age, maturation, and size of the broods maintained for the breeding; and mainly on feeding of artificial feed to the broods. It further depends on stripping of the broods under induced breeding, collection of eggs and milts from them, artificial fertilization of eggs by milt, incubation of zygotes in the locally-made incubation cum hatching trays put inside incubation raceway ponds, and endogenous feeding period of alevins in the locally-made endogenous feeding cum hatching cages put inside hatching raceway ponds.

The zygotes, after passing through eyed-eggs, are hatched into alevins possessing yolk-sac in their throat region. The yolk is utilized for the development process and growth. Because energy is not taken from outside in the form of feed but compensated from the yolk, this period is called endogenous feeding period.

Endogenous feeding period is the procurement of alevins in locally-made cages put inside hatching raceway pond so as to be converted into free swimming fries (FSFs) after final hatching.

The endogenous feeding period is dependent on water temperature (WT) (Bhagat and Barat, 2015) [7]. Both incubation period and endogenous feeding period may collectively be called total incubation period. Because their yolk-sacs are completely absorbed and utilized in the development process and growth, yolk-less hatchlings, also called post-hatchlings, post-larvae or free-swimming fries, after final hatching, accept artificial feed in the form of starter feed or crumble feed by demanding the feed by alternate opening and closing of the gape of their mouth (Pradhan *et al*., 2008) [19]. The demanding of the feed for the purpose of eating is known as exogenous feeding period.

The prime objective of this study was to record the endogenous feeding period of the alevins hatched out from the incubated zygotes stripped from 22 months old male and female 1.0^{+} rainbow trout broods (first spawners) for FSFs release further recording in a sequence, the zygotes (number and size) from the same broods, total incubation period (from zygotes to FSFs), incubation cum hatching period (from zygotes to alevins), calculation of yolk (energy) used in the development process, growth, and formation of yolk-sac until the release of alevins, released alevins (number and size), endogenous feeding cum final hatching period, calculation of yolk (energy) used in the development process and growth until the release of FSFs, and released FSFs (number and size) including survivability and growth of above mentioned stages. Therefore, the study was conducted for 1 year and 7 months from June 2009 to December 2010 on endogenous feeding period of rainbow trout in farmer's raceway ponds at Kakani, Kathmandu, Nepal situated at latitude 27°48' N, longitude 85°15' E and ALT 1550 msl. Water quality parameters like air temperature (AT), WT, pH, DO, free carbon dioxide (FCO), and water discharge (WD) were noted down whenever found necessary to be required and WT, pH, DO, FCO, and WD were recorded as a routine work from June 2010 to December 2010. Hence, incubation period of zygotes (in days) hatched into alevins and endogenous feeding period of alevins (in days) hatched into FSFs obtained from 1 year and 10 months old rainbow trout broods in the raceway ponds at the above mentioned altitude and below mentioned water quality parameters were recorded along with the calculation of yolk (energy) used in development process and growth. Weight and length of broods, stripping, and weight, size, and number of eggs and milt, fertilization and its percentage, incubation of zygotes, cleaning and readjustment, and alevins released into FSFs were also recorded.

2. Materials and Methods

Stoking density was followed according to Rai *et al*. (2008) [20]; water discharge of the raceway pond which was more than the requirement was maintained according to both Basnet *et al*. (2008) [5] and Rai *et al*. (2008) [20]; and ingredients, composition, and feeding of artificial feed was followed according to Basnet *et al*. (2008) [5], Bista *et al*. (2008) [9] and Rai *et al*. (2008) [20].

Method of cultivation of rainbow trout broods, eggs, alevins, and FSFs whether normal, semi-intensive, or intensive during maintenance and breeding based on DO and pH; depth of water whether 80, 90, or 100 cm based on the depth of the raceway pond; water discharge of 0.017, 0.067, or 3.13 L sec^{-1} based on stages of the trout; and stocking density (wt. m^{-2} and number m^{-2}) of the above mentioned stages in the raceway ponds of the size of 5 m × 1 m × 1 m with 5 m × 1 m × 0.9 m volume of water flowing whether 5, 10, or 15 kg m^{-2} and 10, 20, or 30 trout m^{-2} based on method of cultivation; crude protein (CP) percent whether 35, 40, or 45% based on stages of the trout; feeding rate whether 1, 2, 3, 5, 8, 10, 12 or 15% based on body wt., growth, and stages of trout; and feeding frequency (times day^{-1} or times $week^{-1}$) of the artificial feed either crumble or pellet feed for all the stages whether 1, 2, 3, 5, 8, 10 or 12 times day^{-1} and 1 or 2 times $week^{-1}$ based on stages and growth of the trout; criteria for the selection of future brood through observation of external appearance; and criteria for the confirmation of brood, segregated brood, current brood, and gravid brood through observation of external features were carried according to Anonymous (1998) [1], Anonymous (2001) [2], Basnet *et al*. (2008) [5], Bista *et al*. (2008) [9], Rai *et al*. (2008) [20], Roy *et al*. (2008) [21], and Bhagat and Barat (2016) [8].

Fingerlings for the next year experiment (from June 2010 to May 2011) were stocked one year previous from June 2009 to May 2010 so as to obtain them as experimental trout. They were stocked @ 250 g m^{-2} (50 fingerlings m^{-2}) in farmer's owned raceway ponds in WD of 0.083 L sec^{-1} m^{-2} (0.42 L sec^{-1} in the raceway pond) and supplied 45% CP containing artificial feed @ 8-10% of their body weight 8-10 times day^{-1} for three months; in WD of 0.1 L sec^{-1} m^{-2} (0.5 L sec^{-1} in the raceway pond) and fed 45% CP containing artificial feed @ 5-8% of their body weight 6-8 times day^{-1} for another three months; in WD of 0.2 L sec^{-1} m^{-2} (1 L sec^{-1} in the raceway pond) and fed 40% CP containing artificial feed @ 3-5% of their body weight 4-6 times day^{-1} for the next three months; and in WD 0.3 L sec^{-1} m^{-2} (1.5 L sec^{-1} in the raceway pond) and fed 35% CP containing artificial feed @ 2-3% of their body weight 3-4 times day^{-1} for the last three months, i.e., totally for twelve months up to May 2010 (Bhagat and Barat, 2016) [8].

Five months prior to breeding, future broods were selected (through observation of the external appearance); stocked @ 5-10 kg m^{-2} (15-20 trout m^{-2}) in WD 0.42L sec^{-1} m^{-2} (2.08 L sec^{-1} in the raceway pond); and fed 35% CP containing diet @ 2-3% of their live body weight twice day^{-1}. Three months prior to breeding, broods were noticed when they developed initial sign of sexual maturity (through monthly-wise observation of the external features including observation of the vent); stocked @ 5-10 kg m^{-2} (15-20 trout m^{-2}) in WD 0.52 L sec^{-1} m^{-2} (2.6 L sec^{-1} in the raceway pond); and fed 40% CP containing artificial feed @ 2-3% twice day^{-1}. Two months prior to breeding, segregated broods were separated when they developed complete sign of sexual maturity (through monthly-wise observation of the external features including observation of the vent); stocked @ 5-10 kg m^{-2} (15-20 trout m^{-2}) in WD 0.63 L sec^{-1} m^{-2} (3.13 L sec^{-1} in the raceway pond); and fed 45% CP containing diet @ 1-2% twice day^{-1}. Segregated broods separation in two different raceway ponds was done (Basnet *et al*., 2008 and Bhagat and Barat, 2016) [5, 8].

One month prior to breeding, the state of the ripeness of gonads was examined twice a week (through observation of the external features including observation of the vent). In the month of breeding, current broods were confirmed (through observation of the external features including observation of the vent and then with the help of inserting a catheter inside the vent) by collecting eggs and milts from both female and male broods respectively; stocked @ 5-10 kg m^{-2} (15-20 trout m^{-2}) in WD 0.63 L sec^{-1} m^{-2} (3.13L sec^{-1} in the raceway pond); and fed 45% CP containing artificial feed @ 1% 4-5 times $week^{-1}$. Gravid broods were collected when they showed

complete sign of readiness for breeding (when with a gentle pressure on vent a female and male brood started oozing ova and milt respectively). When ready for breeding, gravid broods were counted (manually) and measured (weight by the help of electronic balance and length by measuring scale) and then wiped clean with the help of a towel before stripping (Basnet *et al*., 2008 and Bhagat and Barat, 2016) [5, 8].

Stripping (by applying mild pressure first on lower part of the ovary near the vent and then upward the ovary over the ventral side of the female towards the vent according to Bhagat and Barat, 2016) [8] was done to collect eggs from females and milts from males. Darkness was maintained during stripping to assure more viability of both eggs and milts. Air temperature was also recorded at that time to further ensure the viability. Eggs were collected by simple hand stripping on a sieve with handle (to drip water from the roe) and then cleaned with 0.9% NaCl solution to remove stickiness and further observed (through naked eye and compound microscope both), counted (manually by random sampling taking 5 samples of 1 g each), and measured (diameter with the help of vernier caliper and weight by the help of electronic balance). Milt was also observed (both through naked eye and compound microscope) and counted (under compound microscope using counting chamber slide) (Bhagat and Barat, 2016) [8].

Eggs of two females were fertilized by the milt of 1 male in a container by dry stripping method by stirring them well with the help of a bird's feather and then keeping stand still for one minute. NaCl sol^{n} (0.9%) was poured carefully from the side of the container to remove any dirt, if present, and further cleaning the zygotes to remove stickiness. To do that, the same procedure was repeated again and again until zygotes became transparent. Darkness was maintained during fertilization to ensure more rate of fertilization. AT was also recorded at that time to further assure more rate of fertilization. Zygotes were segregated from unfertilized eggs with the help of colour (zygotes light-coloured and unfertilized eggs dull-coloured) and then counted (manually by random sampling taking 5 samples of 1 g each) to calculate fertilization percentage (fertilized eggs ÷ total number eggs × 100). Spent up broods (1.0^{+} broods or first spawners) were procured and stocked for another year breeding to come (Bhagat and Barat, 2016) [8].

Incubation was done in incubation cum hatching trays. All the procedures were maintained in the evening on the same day of fertilization further maintaining darkness. Darkness was also maintained in the hatchery, as the development would be better. Each tray contained 1000 eggs. Ten such trays were staked together in an atkin. Altogether three atkins were put into an incubation tank kept inside incubation cum hatching raceways. Water discharge was maintained 0.017 L sec^{-1} (for the 1st week), 0.033 L sec^{-1} (for the 2nd week), and 0.05 L sec^{-1} (for the 3rd, 4th and rest weeks) per 10,000 fertilized eggs in the raceway pond according to Basnet *et al*. (2008) [5] and Rai *et al*. (2008) [20]. WT, pH, DO, and FCO were also recorded as a routine work. All the trays were cleaned to remove dead eggs and readjusted weekly-wise to provide equilibrium in all the trays until observing eyed-eggs. Mortality was counted during cleaning and readjustment. Calculations were done to calculate the yolk (energy) used during developmental process and growth until release of alevins along with yolk (g) kept inside yolk-sac. When alevins were released, they were counted (manually by random sampling taking 5 samples each) to calculate their number for survival percentage (alevins released ÷ total number of incubated eggs × 100) and measured (with the help of electronic balance and measuring scale) to know their weight (g), length (cm), and weight of the yolk-sac (g) (Bhagat and Barat, 2016) [8].

Alevins were adjusted in endogenous feeding cum hatching cages put into endogenous cum hatching raceway ponds. WD was maintained 0.067 L sec^{-1} per 10,000 alevins in the raceway according to Basnet *et al*. (2008) and Rai *et al*. (2008). WT, pH, DO, and FCO were also recorded. Darkness was maintained in the hatchery. After passing through endogenous feeding period (in days) based on WT, FSFs would leave the substratum and start free swimming after complete absorption of their yolk-sac. Mortality was counted during the period. Calculations were further done to calculate the yolk (energy) used during developmental process and growth until release of FSFs. When FSFs were released they were counted (manually by random sampling taking 5 samples each) and measured to calculate their number for survival percentage (FSFs released ÷ total number of alevins × 100) and measured (with the help of electronic balance and measuring scale) to know their weight (g) and length (cm). FSFs were then made ready for exogenous feeding period for the feeding of formulated diets (Bhagat and Barat, 2016) [8].

3. Results

In June 2009, i.e., 1 year and 5 months ahead of breeding, 250 fingerlings, each 5 months old, 5.433±0.06g wt., 6.70±0.02cm long, and totaling 1.358 kg were stocked @ 250 g m^{-2}, i.e., 50 fingerlings m^{-2} in WD 0.067-1.0 L sec^{-1} were obtained 225 in number out of 250 confirming 90% survivability and 364.14±18.02 g growth from 5.433±0.06 g further confirming 30.345 g month^{-1} growth as experimental trout, each 1 year 5 months old, 369.57 ± 18.06 g wt., 24.8 ± 0.17 cm long, and totaling 83.15 kg in May 2010, i.e., after 1 year of stocking and 5 months ahead of breeding.

In June 2010, i.e., 5 months ahead of breeding, 200 future broods, each 1 year 5 months old, 375 ± 18.097 g wt., 31.5 ± 0.19 cm long, and totaling 75 kg were stocked @ 7.5 kg m^{-2}, i.e., 20 trout m^{-2} in WD 1.0-2.08 L sec^{-1} were obtained 200 in number out of 200 confirming 100% survivability and 25 g growth in June from 375 ± 18.097 g confirming 25 g month^{-1} growth and 26 g growth in July from 400 ± 18.01 g further confirming 26 g month^{-1} growth as broods, each 1 year 7 months old, 426 ± 16.165 g wt., 33 ± 0.40 cm long, and totaling 85.2 kg, in July 2010, i.e., after 1 year and 2 months of stocking and 3 months ahead of breeding when males developed rough upper surface on pectoral fins and females swollen belly along with slightly lined reddish vent. When maturity of the gonads of broods was checked monthly-wise in June and July, 2010, it was found in increasing trend.

In August 2010, i.e., 3 months ahead of breeding, 200 broods, each 1 year and 7 months old, 426 ± 16.165 g wt., 33 ± 0.40 cm long, and totaling 85.2 kg were stocked @ 8.52 kg m^{-2}, i.e., 20 trout m^{-2} in WD 2.08-2.6 L sec^{-1} were obtained 200 in number out of 200 confirming 100% survivability and 28 g growth from 426 ± 16.165 g further confirming 28 g month^{-1} growth as segregated broods, each 1 year and 8 months old, 454 ± 18.641 g wt., 34 ± 0.39 cm long, and totaling 90.8 kg, in August 2010, i.e., after 1 year and 3 months of stocking and 2 months ahead of breeding when males became bright and brilliant in colour with compressed abdomen and elongated lower jaw, curved upwards like a hook and some males appearing darker in colour, almost black and females comparatively light-coloured than males but with swollen and

enlarged abdomen having slightly reddish vent. When maturity of the gonads of segregated broods was checked monthly-wise in August, 2010, it was found in increasing trend.

In September 2010, i.e., 2 months ahead of breeding, 200 segregated broods, each 1 year and 8 months old, 454 ± 18.641 g wt., 34 ± 0.39 cm long, and totaling 90.8 kg were stocked @ 9.08 kg m^{-2}, i.e., 20 trout m^{-2} were obtained 200 in number out of 200 confirming 100% survivability and 29 g growth in September from 454 ± 18.641 g confirming 29 g $month^{-1}$ and 30 g growth in October from 483 ± 18.01 g further confirming 30 g $month^{-1}$ growth as current broods, each 1 year and 10 months old, 513 ± 17.678 g wt., 36 ± 0.38 cm long, and totaling 102.6 kg, in October 2010, i.e., after 1 year and 5 months of stocking and one week ahead of breeding when males oozed milt on pressing their abdomen and females eggs along with reddish vent. When maturity of the gonads of current broods was checked monthly-wise in September and October, 2010, it was found in increasing trend.

In the 1st week of November 2010, i.e., in the month of breeding, 200 current broods each 1 year and 10 months old, 513 ± 17.678 g wt., 36 ± 0.38 cm long, and totaling 102.6 kg were stocked @ 10.26 kg m^{-2}, i.e., 20 trout m^{-2} were obtained 200 in number out of 200 confirming 100% survivability and 6 g growth from 513 ± 17.678 g further confirming 1 g day^{-1} growth in the 1st week of November as gravid broods, each 1 year 10 months old, 519 ± 19.191 g wt., 36 ± 0.38 cm long, and totaling 103.8 kg, in the 2nd week of November, 2010, i.e., after 1 year and 5 months of stocking and one day before breeding when both males and females developed complete sign of readiness for breeding. When maturity of the gonads of gravid broods was checked weekly-wise in the last day of the 1st week of November, it was found in increasing trend. The gravid broods of 1 year and 10 months were designated as 1.0^{+}broods (first spawners).

On Sunday, 7th November 2010, i.e., on the day of breeding, one hundred 1.0^{+}broods (first spawners), each 1 year and 10 months old, 519 ± 19.191 g wt., 36 ± 0.38 cm long, and totaling 51.9 kg were netted out and stocked @ 10.38 kg m^{-2}, i.e., 20 trout m^{-2} inside happa kept in raceway ponds. Out of 100 gravid broods, which were 1.0^{+}broods (first spawners) and which were put under artificial breeding to know the endogenous feeding period, few gravid broods, when selected and collected before spawning were found to be 519.83 ± 12.243 g wt. and 36.06 ± 0.34 cm long. Among them 18 gravid broods (12 females and 6 males) were selected for the research experiment.

Stripping of 12 female (520±9.785g and 36.18±0.241cm) and 6 male broods (493.5 ± 23.158 g and 35.8 ± 0.79 cm) in the evening time in semi-intensive culture system showed yellow-coloured eggs each 0.0499 ± 0.001 g and 0.303 ± 0.0095 cm (diameter) and cream-coloured milts each ml containing 15 millions spermatozoa. Stripping range was 69-85 g eggs $female^{-1}$ which was 1380-1848 number eggs $female^{-1}$ and 26-41 ml of milt $male^{-1}$ which was 3.90-6.15 billions spermatozoa $male^{-1}$ respectively. Therefore, in total 915 g eggs (76.25 ± 1.538g eggs $female^{-1}$) coming to be 18391 number eggs (1532.58 ± 38.88 number eggs $female^{-1}$) and 204 ml milt (34 ± 2.61 ml milt $male^{-1}$) respectively were collected. 146.65 ± 1.404 g eggs kg^{-1} body wt. (1759.82 g eggs 12 kg^{-1} body wt.) and 2947.42 ± 51.54 number eggs kg^{-1} body wt. (35369 number eggs 12 kg^{-1} body wt.) of females were laid. 1 g eggs contained 20.12 ± 0.38 number eggs (241376 number eggs 12 kg^{-1} and 20115 number eggs kg^{-1} of eggs). Milting (oozing of milts) was 68.53 ± 2.36 ml (411.17 ml milts 6 kg^{-1} body wt. of males).

After stripping, spent up 1.0^{+} (first spawners) broods were managed as future broods for the next year breeding and stocked @ 10 kg m^{-2} (20 trout m^{-2}) maintaining WD 6 L sec^{-1} m^{-2} and fed 45% CP containing artificial pellet feed @ 2-3% of their body weight twice day^{-1}.

At the time of stripping and fertilization, AT was recorded 16.8 ˚C. Artificial fertilization was procured in the evening on Sunday, 7 November 2010 with 70.18 ± 4.05 g zygotes $female^{-1}$ (842.1 g zygotes 12 $females^{-1}$) and 1410.16 ± 94.28 number zygotes $female^{-1}$ (16922 number zygotes 12 $females^{-1}$) ensuring fertilization percentage to be 91.99 ± 0.58%.

WT ranged 9.1-21.5 (17.69 ± 1.85 ˚C), pH 6.7-7.8 (7.24 ± 0.18), DO 7.2-10.1 (8.37 ± 0.49mg L^{-1}), FCO 1.8-4.9 (3.71 ± 0.41 mg L^{-1}) and WD 37-84 (57.57 ± 6.28L sec^{-1}) from June 2010 to December 2010.

Zygotes were transferred in the evening into incubation cum hatching trays put into incubation cum hatching raceway ponds on Sunday, 7 November 2010 maintaining WD 0.033 L sec^{-1} @ 10,000 eggs in WT 13.1 ˚C, pH 8.0, DO 9.8 mg L^{-1}, and FCO 2.9 mg L^{-1}. Cleaning and readjustment of incubation cum hatching trays were done thrice – 1st on Sunday, 14 November 2010; 2nd on Sunday, 21 November 2010; and 3rd on Sunday, 28 November 2010 so as to remove dead eggs. During 3rd cleaning, eyed-eggs were seen confirming hatching to occur after 3-4days. Hatching of fertilized eggs occurred on Thursday, 2 December 2010 after 4 days of last cleaning and readjustment. Incubation period was 25 days (Figure-1) in average temperature of 10.89±0.21 ˚C day^{-1} and cumulative temperature (sum total of WT of 25 days) of 272 ˚C with hatching of yolk-sac alevins (survivability 41.82 ± 3.16%), each 0.0365 ± 0.0016 g and 1.46 ± 0.086 cm with 0.0136 ± 0.0012 g yolk-sac (Table-1). Yolk (energy) used during the developmental process, growth, and formation of yolk-sac (also keeping yolk inside it) due to conversion and change of zygotes into alevins was 0.01342 g (26.89%), 0.0365 g (73.15%), and 0.0136 g (27.26%) respectively. Further, yolk-sac was 37.26% of alevins.

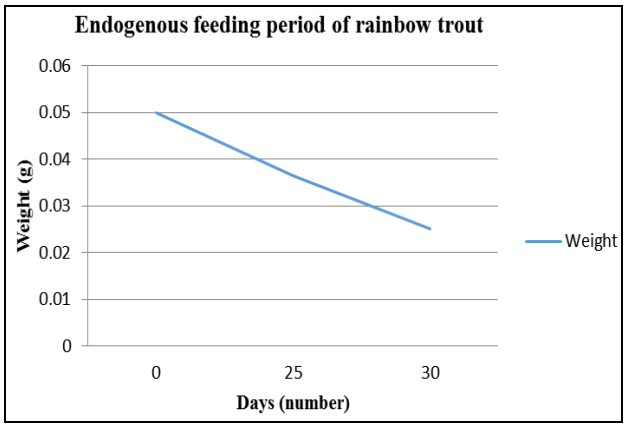

Fig 1: Conversion and change of zygotes to alevins to free swimming fries (decreasing in weight due to increasing in days)

Alevins were transferred in the evening into endogenous feeding cum hatching cages put into endogenous feeding cum hatching raceway ponds on Thursday, 2nd December, 2010 maintaining WD 0.033 L sec^{-1} in WT 9.1 ˚C, pH 8.0, DO 10.1 mg L^{-1}, and FCO 1.8 mg L^{-1}. Hatchability was noticed on Tuesday, 7 December 2010 after 5 days (Figure-1). Hence,

endogenous feeding period was 5 days in WT of 9.1 °C releasing FSFs (survivability 76.92 ± 4.91%) each 0.025 ± 0.0007 g and 1.65 ± 0.083 cm (Table-1) ready for exogenous feeding. Yolk (energy) of the yolk-sac used during the developmental process and somatic growth due to conversion and change of alevins into FSFs was 0.0115 g (84.56%) and 0.0021 g (15.44%) respectively. Further, developmental process of alevins into FSFs was 31.51% of alevins and growth 5.75% (Table-1).

Table 1: Comparison in the size (weight decreasing and length increasing) of zygotes, alevins and free swimming fries

Zygotes		Alevins			Free swimming fries		Remarks
Weight (g)	Diameter (cm)	Weight (g)	Length (cm)	Yolk-sac (g)	Weight (g)	Length (cm)	
0.047	0.25	0.039	1.70	0.016	0.027	1.8	Tray A
0.050	0.31	0.033	1.30	0.012	0.024	1.5	Tray B
0.051	0.25	0.042	1.80	0.018	0.027	2.0	Tray C
0.045	0.33	0.037	1.50	0.015	0.024	1.7	Tray D
0.049	0.34	0.044	1.90	0.0 20	0.029	2.1	Tray E
0.054	0.29	0.036	1.40	0.012	0.024	1.6	Tray F
0.046	0.30	0.032	1.20	0.010	0.023	1.4	Tray G
0.047	0.29	0.029	1.10	0.009	0.023	1.3	Tray H
0.053	0.28	0.041	1.50	0.014	0.026	1.7	Tray I
0.055	0.32	0.032	1.20	0.010	0.023	1.4	Tray J
0.049	0.35	–	–	–	–	–	–
0.053	0.33	–	–	–	–	–	–
0.04992	0.0303	0.0365	1.46	0.0136	0.025	1.65	Average

4. Discussion

Rainbow trout breed from September to February in India (Santhanam *et al*., 1999) [22] and November to February in Nepal (Basnet *et al*., 2008) [5]. Successful breeding of rainbow trout, in this research work, in November by induced breeding resembled both Santhanam *et al*. (1999) [22] and Basnet *et al*. (2008) [5] further confirming artificial method of propagation to be the successful method of breeding. Rainbow trout can breed twice but breeds only once during breeding season in Nepal (Basnet *et al*., 2008 and Rai *et al*., 2008) [5, 20] because breeding twice gives poor spawning results with mortality of broods, incubated eggs, alevins, and FSFs, however, breeding only once gives good spawning results with comparatively more survivability of broods, incubated eggs, alevins, and FSFs. According to Crandell and Gall (1993) [11], a male rainbow trout spawns at 1 yr and a female at 2 yrs; according to Rai *et al*. (2008) [20], rainbow trout breed after attaining 2 yrs; and according to Basnet *et al*. (2008) [5], it matures at the age of 2-3 yrs, however, a female rainbow trout spawns best at the age of 4-7 years while a male at 3-6 years. However, 1.0^{+}broods (first spawners) and 2.0^{+}broods (second spawners) are used for breeding in Nepal at small farmers' level because of their constraints in brood management for such a long time of 3 to 6 years.

Breeding performance and success of rainbow trout culture highly depend on selection, management, age, and maturation of brood, disease surveillance, feeding, and water quality (Basnet *et al*., 2008) [5]. Good selection of brood is one of the important aspects to increase the rate of hatchability and decrease rate of mortality of offspring, hence, quality, quantity and matured eggs and milt can be obtained by careful selection of broods (Basnet *et al*., 2008) [5]. Further, according to Basnet *et al*. (2008) [5], 1.0^{+}broods (first spawners), should be selected based on external appearance and weight. So, most of the experimental broods (200 out of 225) were selected as future broods according to Basnet *et al*. (2008)[5], on the basis of general health condition, absence of deformities, good external appearance, rapid growth, good colouration, prompt activity, swiftness of reaction to stimuli and weight. Segregated broods were put into separate raceways to increase sexual affinity between females and males so as to increase quality and quantity of eggs and milts respectively.

Stocking density, CP (%) in artificial feed, feeding rate (%), feeding frequency (times day^{-1}), stocking duration (months/year) and WD (L sec^{-1} m^{-2}) maintained for fingerlings total 1.358 kg each 5.433 ± 0.078 g wt. and 6.70 ± 0.03 cm long stocked @ 250g m^{-2} (50 fingerlings m^{-2}) following Marcel (1995) [16] and Basnet *et al*. (2008) [5] and obtaining them after 1 year (in May, 2010) as experimental trout total 83.15 kg each 369.57 ± 18.06 g wt. and 24.8 ± 0.17 cm long with 90% survivability and 30.345 g month^{-1} (for 12 months) of growth; maintained for future broods total 75 kg each 375 ± 18.097g wt. and 31.5± 0.19 cm long stocked @ 7.5 kg m^{-2} (20 trout m^{-2}) and obtaining them after 2 months (in July 2010) as broods total 85.2 kg each 426 ± 16.165 g wt. and 33 ± 0.40 cm long with 100% survivability and 26.5 g month^{-1} (for 2 months) growth; maintained for broods total 85.2 kg each 426 ± 16.165 g wt. and 33 ± 0.40 cm long stocked @ 8.52 kg m^{-2} (20 trout m^{-2}) and obtaining them after 1 month (in August, 2010) as segregated broods total 90.8 kg each 454 ± 18.641 g wt. and 34 ± 0.39 cm long with 100% survivability and 28 g month^{-1} (for 1 month) growth; maintained for segregated broods total 90.8 kg each 454 ± 18.641 g wt. and 34 ± 0.39 cm long stocked @ 9.08 kg m^{-2} (20 trout m^{-2}) and obtaining them after 2 months (in October, 2010) as current broods total 102.6 kg each 513 ± 17.678 g wt. and 36 ± 0.38 cm long with 100% survivability and 29.5 g month^{-1} (for 2 months) growth; maintained for current broods total 102.6 kg each 513 ± 17.678 g wt. and 36 ± 0.38 cm long stocked @ 10.26 kg m^{-2} (20 trout m^{-2}) and obtaining them after 1 week (in the 1st week of November 2010) as gravid broods total 103.8 kg each 519 ± 19.191g wt. and 36 ± 0.38 cm long with 100% survivability and 1 g day^{-1} growth; and maintained for all the gravid broods total 103.8 kg each 519 ± 19.191 g wt. and 36 ± 0.38 cm long stocked @ 10.38 kg m^{-2} (20 trout m^{-2}) and obtaining them on the day of breeding as 1.0^{+}broods (first spawners) total 51.9 kg each 519 ± 19.191 g wt. and 36 ± 0.38 cm long gave similar results like Basnet *et al*. (2008) [5] because experimental trout management, future brood selection, and broods, segregated broods, current broods, and gravid broods confirmation were done accordingly. According to Bista *et al*. (2008) [9], survivability of broods was 95-97% whereas it was 100% in the present work. It

might be due to proper management in the present work. The growth of fingerlings to gravid broods was similar to Marcel (1995) [16], Anonymous (2006) [3], Basnet *et al*. (2008) [5], Joshi *et al*. (2008) [14], Rai *et al*. (2008) [20], and Swar (2008) [25].

Yellow colour of eggs was due to the carotenoids present in the feed (Roy *et al*., 1999) [21]. According to Basnet *et al*. (2008) [5], eggs vary from 3-5mm in diameter. Colour and size of eggs resembled Rai *et al*. (2008) [20] and Basnet *et al*. (2008) [5] respectively. According to Martiyshev (1983) [17], the older brood generally lays larger-sized and higher number of eggs and according to Morrissy (1973) [18], a 3-4 years female lays 3000-3500 number eggs kg^{-1} body wt. Further, according to Schlenk and Kahmann (1990) [24], there should be 20 millions spermatozoa ml^{-1} milt. Less number of eggs (2947 eggs in number) each 3.03 mm in diameter and less numbers of spermatozoa (15 millions ml^{-1}milt) in the present work was due to the age of broods being 1 yr. and 10 months only.

Dark condition was created during stripping to ensure more viability and survivability of eggs and sperms; during fertilization to ensure more fertilization percentage of eggs; during incubation to ensure more survival of eggs so as to release alevins; and during hatchability to ensure more survival of alevins releasing free swimming fries (FSFs). Therefore, stripping, fertilization, incubation and hatchability were done in the evening.

One male supplied required milt for the fertilization of the eggs of two females after stripping because according to Basent *et al.* (2008) [5], one male can supply enough milt for the fertilization of eggs of two females. Hence, fertilization was 2:1::eggs:milt also reported by Basnet *et al*., 2008) [5].

Rainbow trout mostly requires glacier water or clean cold spring water for its successful breeding (Basnet *et al.*, 2008) [5]. The water in the raceway ponds situated at the ALT of 1550 msl, in the present investigation, was supplied from permanent and dependable WR fed with perennial spring-fed stream. The prerequisite for rainbow trout cultivation is adequate volume of cold water below 20 ˚C because feed consumption decreases when WT increases above 20 ˚C, resulting into slow growth and eventually death, if exposed to higher (always more than 20 ˚C) WT for a longer period (Rai *et al*., 2008) [20]. What so ever may be, rainbow trout require WT 0-25 ˚C (Swar, 2008) [25] but according to Yamazaki (1991) [27], it grows well in WT 10-18 ˚C, however, according to Anonymous (2001) [2], its best growth in Nepal occurs in WT 16-18 ˚C. According to Rai *et al*. (2008) [5], suitable WT for rainbow trout spawners for breeding and incubation is 9 to 14 ˚C. In the present investigation, WT during breeding and incubation (in November and December) in the present work was 9.1-13.1 ˚C.

The preferable pH for rainbow trout is 6.5-8.0 with optimum value 7.0-7.5 (Rai *et al*., 2008) [20] for semi-intensive farming because at higher pH levels, relatively low levels ammonia (NH_3) can be dangerously toxic (Bromage and Shephard, 1990 and Sedgwick, 1985) [10, 23]. According to Huet (1975) [13], rainbow trout require DO above 7.0 mg L^{-1}; according to Rai *et al*. (2008) [20], it requires DO more than 7.0 mg L^{-1}; and according to Basnet *et al*. (2008) [5], its brood requires cold, clean and high DO containing water of 7.0-7.5 mg L^{-1} for normal trout culture and 10-11 mg L^{-1} for intensive culture for proper ripening of gonads and successful hatching of alevins and FSFs because according to Anonymous (1998) [1] the growth is retarded and the trout may die, if exposed to DO below 7.0 mg L^{-1}. Rainbow trout require FCO below 20 mg L^{-1} (Lawson, 2011) [15]. So, induced breeding under semi-intensive culture was done in the present work because pH was 6.7-7.9 and DO below 11 mg L^{-1}. Hence, parameters like WT, pH, DO and FCO, in the present investigation, were suitable for breeding and WD was such that it could be maintained from 0.017 to 3.13 L sec^{-1} as per requirement as mentioned above in the materials and methods.

As a rule of thumb, hatching of eyed-eggs into alevins would have been occurred between 25 to 30 days after fertilization if cumulative WT might have been 225-275 ˚C, DO 6-8mg L^{-1} and WD of 0.034 L sec^{-1} for 10,000 fertilized eggs (Rai *et al*., 2008) [20]. In the present work, the cumulative WT was 272 ˚C in the hatching period of 25 days. Alevins or hatchlings growing period of rainbow trout is from March to April when egg is surrounded by yolk-sac (denoting endogenous feeding period) nourishing the developing egg nucleus (Watson, 1993) [26]. In the present investigation, alevins growing period in Nepal is from November to December because of the presence of suitable WT during the period.

Alevins or hatchlings having yolk-sac obtained from well-fed 3 years matured female and male brood show more survival, growth, activeness, and more yolk-size of alevins than those not well fed but of the same age and also to those of 2 years of age. Similarly, alevins obtained from well-fed 2 years matured female and male brood show more survival, growth, activeness, and more yolk-size of alevins than those not well fed but of 3 years age (Martyshev, 1983) [17]. Water quality, brood maintenance, and artificial feed play an important role in achieving higher hatchability (Basnet *et al*., 2008) [5]. Because hatchability was survivability, therefore, it was less being 41.82 ± 3.16% probably due to the age and size of the brood.

According to Frost and Brown (1967) [12], hatching period would have been depended on WT and might have been taken place in 27-45 days when WT would have been remained 10-12 ˚C. The fertilized eggs hatch within 27-30 days at 9-14 ˚C (Anonymous, 2006) [3] and 20-30 days depending on water temperature (Rai *et al*., 2008) [20]. Hatching occurred after 27-35 days in Trishuli when WT ranged 10-13 ˚C whereas it took 25-30 days in Godawari when WT ranged 11-14 ˚C (Basnet *et al*., 2008)5. High WT during spawning season claimed deformed alevins (Basnet and Silwal, 1996) [6]. No deformed alevins was found as the WT was quite suitable. The incubation period was 25 days in WT of 10.89±0.21 ˚C day^{-1} in the present work.

The yolk-sac got absorbed within 5-7 days (Martyshev, 1983)17 and 7-18 days (Basent *et al*., 2008) [5]. The yolk-sac of yolk-sac fry of rainbow trout weighing 0.08 g was found absorbed in 2 weeks (Rai *et al*., 2008) [20]. Endogenous feeding period depends on WT (Bhagat and Barat, 2015) [7]. It was 5 days in WT of 9.1 ˚C the present investigation.

After hatching, alevins are carefully removed from the trays into the freely-hanging hatching cages where running water is maintained by protecting them from bright light. Water supply rate in the freely-hanging hatching cages are at the rate of 0.3-0.5 L sec^{-1} for 10,000 alevins. Hatchability was low in Godawari and Trishuli than in Kakani and Rasuwa. On the first day, immediately after hatching, alevins are 1.3-1.8 cm in length and 40-50 mg in weight (Basnet *et al*., 2008) [5]. A larva, also known as alevin, measures about 1.3-1.8 cm in length and 50-80mg in weight. Out of the total weight of alevin, yolk-sac constitutes about 50-60% weight (Rai *et al*., 2008). In the present work, zygotes which were converted

into alevins were 1.46 ± 0.086 cm in length and 0.0365 ± 0.0016 g in wt. with 0.0136 ± 0.0012 g wt. of yolk-sac. So, conversion and change of yolk of zygotes to alevins was 26.89% for developmental process, 73.15% for growth, and 27.26% for yolk-sac which was 36.26% of alevins. Similarly, in the present work, alevins which were converted into FSFs were 1.65cm in length and 0.025 g wt. Therefore, conversion and change of yolk of yolk-sac of alevins to FSFs was 84.56% for developmental process and 15.44% for growth which respectively was 31.51% of alevins for developmental process and 5.75% of alevins for growth. Hence, conversion and change of zygotes to alevins to FSFs was comparatively less because of the small age and size of the brood.

As a rule of thumb, hatching of FSFs would have been occurred between 7 to 30 days after incubation of alevins if WT might have been 9-10 °C, DO 10-11 mg L^{-1}, pH 7.5-8.5 and WD of 0.05 L sec^{-1} for 10,000 alevins along with proper shelter. However, alevins weighing 0.08 g become FSFs in 2 weeks (Rai *et al*., 2008) [20]. In the present work, total incubation period from zygotes to FSFs was 30 days which was due to the moderate WT of 9.1-13.1 °C. Results confirmed the endogenous feeding period of 5 days in WT of 9.1 °C in the present investigation. Afterwards, FSFs start feeding called exogenous feeding period.

5. Conclusion

Results of 1 year and 10 months old rainbow trout broods confirmed the whole development period (incubation period + endogenous feeding period) of zygotes to FSFs being 30 days from 7 November to 2 December 2010 depending on WT in the WT of 13.1 °C. Hence, conversion and change of the yolk of zygotes into alevins for developmental process, growth, and formation of yolk-sac and further, yolk of the yolk-sac of alevins into free swimming fries for developmental process and growth were comparatively less because of the small age and size of the broods. Further, the incubation period of zygotes to alevins was 25 days in temperature of 10.89 ± 0.21 °C day^{-1} and cumulative temperature of 272 °C with 41.82 ± 3.16% survivability releasing alevins each 1.46 ± 0.086 cm and 0.0365 ± 0.0016 g with 0.0136 ± 0.0012 g yolk-sacs. Furthermore, the endogenous feeding period of alevins to FSFs was 5 days in WT of 9.1 °C with 76.92 ± 4.91% survivability releasing the FSFs each 1.65 ± 0.083 cm and 0.025 ± 0.0007 g which were ready for exogenous feeding period.

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