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Endogenous Feeding Period of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum), in the Raceways of Kathmandu, Nepal

Rakesh Prasad Bhagat[1, 2,*] and Sudip Barat[2]

[1]Department of Zoology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu-44600, Nepal

[2]Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, Siliguri-734 013, District Darjeeling, West Bengal, India

*Corresponding Author E-mail: rakesh.bhagat2009@gmail.com

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ABSTRACT

Endogenous feeding period of 2 years and 10 months old 2.0+ broods (second spawners) of rainbow trout, Oncorhynchus mykiss (Walbaum), was recorded in the farmers' raceways. Twelve female (39.56 ± 0.25 cm and 697 ±11.15 g) and six males (39.12 ± 0.41 cm and 690.5 ± 26.46 g) were bred obtaining 1404g eggs and 309 ml milts respectively. Eggs were yellow and milt cream-coloured. Each egg was 0.405 ± 0.0091cm in diameter and 0.6025 ± 0.001g in weight and each millimetre of milts comprised 20 millions spermatozoa. Fertilization was done by dry stripping method obtaining 1299.2 g (21562 numbers) zygotes thus, ensuring fertilization percentage to be 92.56 ± 0.45%. Conversion and change of the yolk of zygotes into sac-fries for development, growth, and formation of yolk-sac and further, yolk of the yolk-sac of sac-fries into free swimming fries for development and growth were comparatively less due to small-sized zygotes because of less age and small size of the broods. The incubation period was 29 days in water temperature of 9.34 ± 0.17°C day^{-1} and cumulative water temperature (sum total of water temperature of 29 days) of 269.8°C with hatching of sac-fries (survivability 44.03 ± 2.42%), each 1.73 ± 0.01 cm and 0.0434 ± 0.002 g with 0.0163 ±0.001 g yolk-sac. The endogenous feeding period was 7 days in water temperature of 8.6°C liberating free swimming fries (survivability 78.26 ± 2.35%), each 1.92 ± 0.1 cm and 0.0406 ± 0.002 g. Total incubation period was 36 days in water temperature of 8.6-11.2°C.

Key words: *Zygotes, Conversion and change, Sac-fries, Endogenous feeding period, Free swimming fries.*

INTRODUCTION

Incubation of zygotes of rainbow trout, *Oncorhyncus mykiss* (Walbaum, 1792)[4] to be converted into sac-fries (SFs) requires conversion and change of zygotes into SFs in which length of the zygotes increase and weight decrease because of the increasing days. Similarly, endogenous feeding period of SFs into free swimming fries (FSFs) requires conversion and change of SFs into FSFs in which length increases and weight of the yolk-sac decreases because of the increasing days.

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Both incubation period and endogenous feeding period may collectively be denoted as total incubation period in which conversion and change of zygotes into SFs for development, growth, and formation of yolk-sac and further, yolk of the yolk-sac of SFs into FSFs for development and growth respectively occurs with increasing in length and decreasing in weight due to increasing in days.

The ultimate objective of this study was to record the endogenous feeding period of the SFs hatched out from the incubated zygotes stripped from 2 years and 10 months old male and female 2.0^{+} rainbow trout broods (second 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 SFs), calculation of yolk (energy) used in the development, growth, and formation of yolk-sac until the release of SFs, released SFs (number and size), endogenous feeding cum final hatching period, calculation of yolk (energy) used in the development 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 2010 to December 2011 on endogenous feeding period of rainbow trout in farmer's raceways at Kakani, Kathmandu, Nepal situated at latitude 27°48' N, longitude 85°15' E and altitude (ALT) 1550 msl. Physico-chemical properties like air temperature (AT), water temperature (WT), power of hydrogen ion concentration (pH), dissolved oxygen (DO), free carbon dioxide (FCO), and water discharge (WD) were recorded whenever found necessary however, WT, pH, DO, FCO, and WD were noted down as a routine work from June 2011 to December 2011. Hence, incubation period of zygotes (in days) hatched into SFs and endogenous feeding period of SFs (in days) hatched into FSFs obtained from 2 years and 10 months old rainbow trout broods in the raceways at the above mentioned ALT and below mentioned physico-chemical properties were recorded along with the calculation of yolk (energy) of zygotes and yolk-sac of SFs used in the development and growth. Length and weight of broods; stripping of broods; size, weight, and number of eggs; volume of milt and number of spermatozoa; fertilization and its percentage; incubation of zygotes; cleaning and readjustment of zygotes; zygotes released into SFs; and SFs released into FSFs were also studied.

MATERIALS AND METHODS

Stoking density was followed according to [22]; WD of the raceway which was more than the requirement was maintained according to both[6] and [22]; and ingredients, composition, and feeding of artificial feed was followed according to[6, 11, 22, 9, and 10]. Method of cultivation of rainbow trout broods, eggs/zygotes, SFs, 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; WD whether 0.017, 0.067, or 3.13 L sec^{-1} based on stages of the rainbow trout; and stocking density (number m^{-2} and wt. m^{-2}) of the above mentioned stages in the raceways of the size of 5 m × 1 m × 1 m with 5 m × 1 m × 0.9 m volume of WD whether 10, 20, or 30 trout m^{-2} and 5, 10, or 15 kg 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[2, 1, 6, 11, 22, 23, 9, and 10].

Spent up broods for the next year experiment (from June 2011 to December 2011) were stocked 7 months previous from November 2010 to May 2011 so as to obtain them as experimental brood. They were stocked @ 15-20 m^{-2} (5-10kg m^{-2}) in farmer's owned raceways maintaining water changing 4 times day^{-1} in WD of 2.08L sec^{-1} m^{-2} (0.42L sec^{-1} in the raceway) and fed 45% CP containing diet @ 2-3% of their body weight two times day^{-1} to obtain them as future broods[9 and 10].

Five months prior to breeding, future broods were selected (through observation of the external appearance); stocked @ 15-20 m^{-2} (5-10 kg m^{-2}) in WD 0.42L sec^{-1} m^{-2} (2.08 L sec^{-1} in the raceway); 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 @ 15-20 m^{-2} (5-10 kg m^{-2}) in WD 0.52 L sec^{-1} m^{-2} (2.6 L sec^{-1} in the raceway); 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 @ 15-20 m^{-2} (5-10 kg m^{-2}) in WD 0.63 L sec^{-1} m^{-2} (3.13 L sec^{-1} in the raceway); and fed 45% CP containing diet @ 1-2% twice day^{-1}. Segregated broods separation in two different raceways was done to increase sexual affinity between male and female broods so that they may increase quality and quantity of eggs and milts respectively[6, 9, and 10].

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 @ 15-20 m^{-2} (5-10 kg m^{-2}) in WD 0.63 L sec^{-1} m^{-2} (3.13L sec^{-1} in the raceway); 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[6, 9, and 10].

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[10]) was done to collect eggs from females and milts from males. AT was recorded during stripping to know viability. Darkness was also maintained at that time to assure more viability of both eggs and milts. Eggs were collected by simple hand stripping on a sieve with handle (to drip water from the roe) and then cleaned with 0.9% sodium chloride solution to remove stickiness and further observed (through both naked eye and compound microscope), measured (diameter with the help of vernier calliper and weight by the help of electronic balance) and counted (manually by random sampling taking 5 samples of 1 g each). Milt was also observed (both through naked eye and compound microscope) and counted (under compound microscope using counting chamber slide) following[10 and 27].

Eggs of two females were fertilized by the milt of one male in a container following dry stripping method by stirring them well with the help of bird's feather and then keeping stand still for one minute. 0.9% sodium chloride solution 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 clear. AT was recorded at that time to know rate of fertilization. Darkness was also maintained during fertilization to ensure 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 (total number of fertilized eggs ÷ total number eggs × 100). Spent up broods (2.0^+ broods or second spawners) were maintained and then stocked for the next year breeding to come[10].

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 zygotes. Ten such trays were staked together in an atkin. Altogether three such atkins were put into an incubation tank kept inside incubation cum hatching raceways. WD 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 zygotes in the raceway according to[6 and 22]. WT, pH, DO, and FCO were also recorded as a routine work from June 2011 to December 2011. All the trays were cleaned to remove dead zygotes 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 and growth until release of SFs along with yolk (g) kept inside yolk-sac. When SFs were released, they were counted (manually by random sampling taking 5 samples each) to calculate their number for survival percentage (SFs released ÷ total number of incubated zygotes × 100) and measured (with the help of measuring scale and electronic balance) to know their length (cm), weight (g), and weight of the yolk-sac (g)[10].

SFs were adjusted in endogenous feeding cum hatching cages put into endogenous cum hatching raceways. WD was maintained 0.067 L sec^{-1} per 10,000 SFs in the raceway according to [6 and 22]. 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 freely 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 development 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 SFs × 100) and measured (with the help of measuring scale and electronic balance) to know their length (cm) and weight (g). FSFs were then made ready for exogenous feeding period for the feeding of formulated diets[10].

RESULTS

In November 2010, i.e., 1 year former to breeding, 150 spent up broods, each 1 year and 10 months old, 36.06 ± 0.28 cm long, 442.611 ± 7.979 g wt., and totaling 66.392 kg were stocked @ 15 m^{-2}, i.e., 6.639 kg m^{-2} in WD 2.08 L sec^{-1} (0.42 L sec^{-1} m^{-2}) were obtained 150 in number out of 150 confirming 100% survivability with growth of 0.82 cm long, 107.389 g wt., and totaling 16.108 kg further confirming 0.12 cm long, 15.341 g wt., and totaling 2.301 kg growth $month^{-1}$ as experimental brood further taken as future broods, each 2 years 5 months old, 36.88 ± 0.25 cm long, 550 ± 8.939 g wt., and totaling 82.5 kg in May 2011, i.e., 5 months former to breeding and 7 months after stocking.

In June 2011, i.e., 5 months former to breeding, 150 future broods, each 2 years 5 months old, 36.88 ± 0.25 cm long, 550 ± 8.939 g wt., and totaling 82.5 kg were stocked @ 15 m^{-2}, i.e., 8.25 kg m^{-2} in WD 2.08-2.6 L sec^{-1} (0.42-0.52 L sec^{-1} m^{-2}) were obtained 150 in number out of 150 confirming 100% survivability with growth of 0.09 cm long, 29 g wt., and totaling 4.35 kg $month^{-1}$ in June and with growth of 0.11 cm long, 30 g wt., and 4.5 kg $month^{-1}$ in July as broods, each 2 years 7 months old, 37.08 ± 0.25 cm long, 609 ± 4.909

g wt., and totaling 91.35 kg, in July 2011, i.e., 3 months former to breeding and 9 months after stocking 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, 2011, it was found in increasing trend.

In August 2011, i.e., 3 months former to breeding, 150 broods, each 2 years and 7 months old, 37.08 ± 0.25 cm long, 609 ± 4.909 g wt., and totaling 91.35 kg were stocked @ 15 m^{-2}, i.e., 9.135 kg m^{-2} in WD 2.08-2.6 L sec^{-1} (0.42-0.52 L sec^{-1} m^{-2}) were obtained 150 in number out of 150 confirming 100% survivability with growth of 0.53 cm long, 31 g wt., and totaling 4.65 kg $month^{-1}$ as segregated broods, each 2 years and 8 months old, 38.l4 ± 0.26 cm long, 640 ± 6.557 g wt., and totaling 96 kg, in August 2011, i.e., 2 months former to breeding and 10 months after stocking 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 2011, it was found in increasing trend.

In September 2011, i.e., 2 months former to breeding, 150 segregated broods, each 2 years and 8 months old, 38.l4 ± 0.26 cm long, 640 ± 6.557 g wt., and totaling 96 kg were stocked @ 15 m^{-2}, i.e., 9.6 kg m^{-2} in WD 2.6-3.13 L sec^{-1} (0.52-0.63 L sec^{-1} m^{-2}) were obtained 150 in number out of 150 confirming 100% survivability with growth of 1.4 cm long, 32 g in wt., and 4.8 kg $month^{-1}$ in September and 1.5 cm long, 33 g wt., and 4.95 kg in October as current broods, each 2 years and 10 months old, 41.04 ± 0.35 cm long, 700 ± 24.988 g wt., and totaling 105 kg, in October 2011, i.e., one week former to breeding and 1 year after stocking 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 2011, it was found in increasing trend.

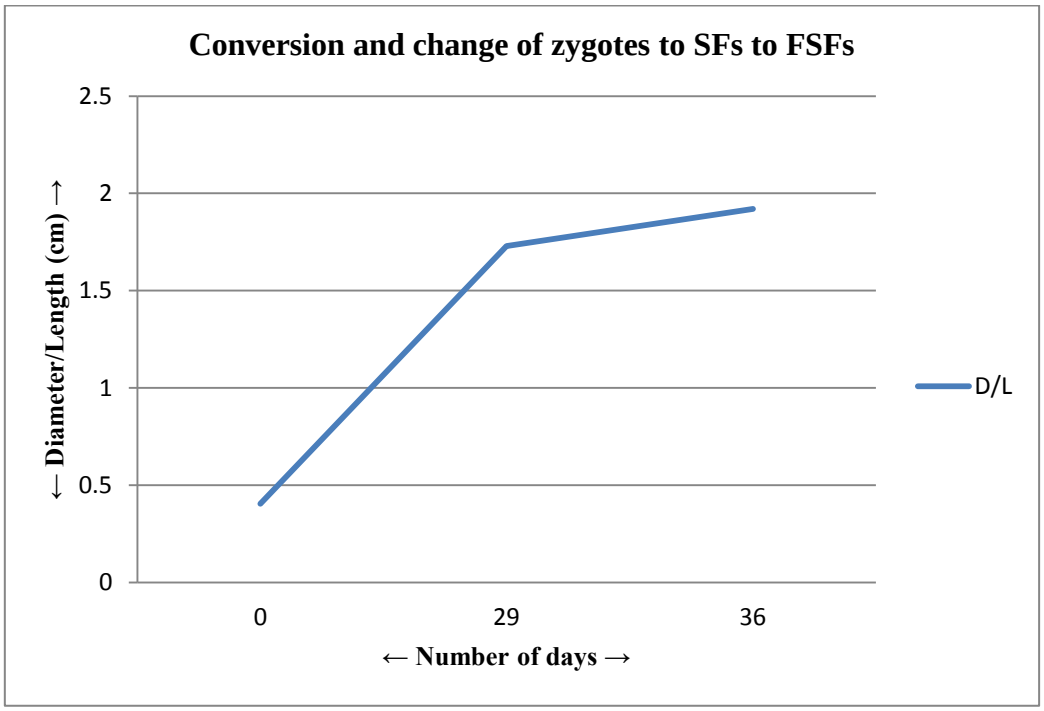

Fig. 1: Total incubation period (increasing in length with increasing days)

In the 1st week of November 2011, i.e., in the month of breeding, 150 current broods each 2 years and 10 months old, 41.04 ± 0.35 cm long, 700 ± 24.988 g wt., and totaling 105 kg were stocked @ 15 m^{-2}, i.e., 10.5 kg m^{-2} in WD 2.6-3.13 L sec^{-1} (0.52-0.63 L sec^{-1} m^{-2}) were obtained 150 in number out of 150 confirming 100% survivability with growth of 0.04 cm long, 12 g in wt., and totaling 1.8 kg further confirming 0.003 cm long, 1 g wt., and totaling 0.15 kg growth day^{-1} in the 1st and 2nd weeks of November as gravid broods, each 2 years 10 months old, 41.08 ± 0.41 cm long, 712 ± 19.404 g wt., and totaling 106.8 kg, in the 3rd week of November, 2011, i.e., one day former to breeding and 1 year after stocking 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 2nd week of November, it was found in increasing trend. The gravid broods of 2 years and 10 months were designated as 2.0^{+}broods (second spawners).

On Tuesday, 15th November 2011, i.e., on the day of breeding, 50 gravid broods, each 2 years and 10 months old, 41.08 ± 0.41 cm long, 712 ± 19.404 g wt., and totaling 35.6 kg were netted out and stocked @ 10 m^{-2}, i.e., 10.12 kg m^{-2} inside happa kept in raceways. Out of 50 gravid broods, which were 2.0^{+}broods (second 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 39.41 ± 0.21 cm long, 694.833 ± 10.555 g wt. and totaling 34.742 kg. Among them 18 gravid broods (12 females and 6 males) were selected for the research experiment.

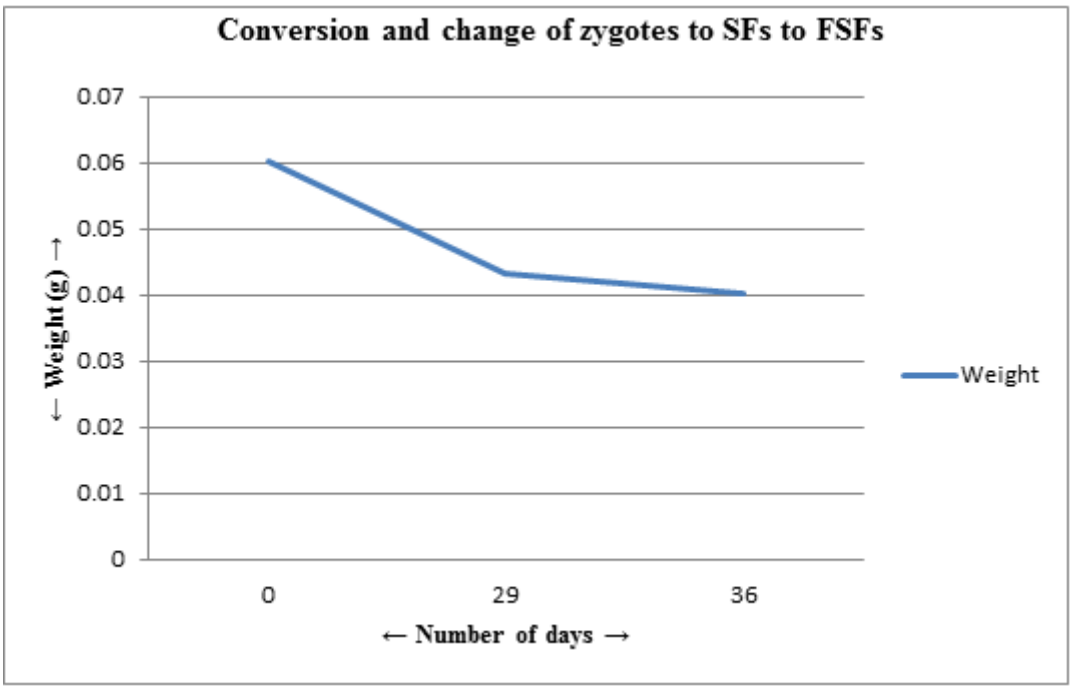

Fig. 2: Total incubation period (decreasing in weight with increasing days)

Stripping of 12 female (39.56 ± 0.25 cm and 697 ± 11.154 g) and 6 males (39.12 ± 0.41 cm and 690.5 ± 26.456 g) in the evening time in semi-intensive culture system showed yellow-coloured eggs each 0.405 ± 0.009 cm (diameter) and 0.06025 ± 0.001 g and cream-coloured milt each ml containing 20 millions spermatozoa. Stripping range was 94-132 g eggs $female^{-1}$ which was 1774-2000 number eggs $female^{-1}$ and 39-62 ml milt $male^{-1}$ which was 7.80-12.40 billions spermatozoa $male^{-1}$ respectively. Therefore, in total 1404 g eggs (117 ± 3.686 g eggs $female^{-1}$) coming to be 23321 number eggs (1943.42 ± 30.727 number

eggs female^{-1}) and 309 ml milt (51.5 ± 3.78 ml milt male^{-1}) respectively were collected. 167.418 ± 2.882 g eggs kg^{-1} body wt. (2009.016 g eggs 12 kg^{-1} body wt.) and 2780.92 ± 24.993 number eggs kg^{-1} body wt. (33371 number eggs 12 kg^{-1} body wt.) of females were laid. 1 g eggs contained 16.6659 ± 0.003 number eggs (199991 number eggs 12 kg^{-1} and 20115 number eggs kg^{-1} of eggs). Milting was 68.53 ± 2.36 ml milts (411.17 ml milt 6 kg^{-1} body wt. of males).

Table 1: Comparison in the size of zygotes, sac-fries and free swimming fries (weight decreasing and length increasing with increasing days) during total incubation period

S.No.	Zygotes		Sac-fries			Free swimming fries		Remarks
	Diameter (cm)	Weight (g)	Length (cm)	Weight (g)	Weight of yolk-sac (g)	Length (cm)	Weight (g)	
1.	0.38	0.057	1.4	0.039	0.012	1.6	0.035	Tray A
2.	0.41	0.062	1.7	0.043	0.014	1.9	0.039	Tray B
3.	0.42	0.059	1.8	0.044	0.018	1.95	0.041	Tray C
4.	0.43	0.064	1.3	0.035	0.011	1.55	0.033	Tray D
5.	0.43	0.064	1.75	0.044	0.017	1.9	0.04	Tray E
6.	0.39	0.058	2	0.045	0.019	2.15	0.047	Tray F
7.	0.36	0.056	1.5	0.04	0.014	1.75	0.038	Tray G
8.	0.35	0.053	2.3	0.054	0.23	2.5	0.049	Tray H
9.	0.44	0.066	1.45	0.039	0.013	1.65	0.36	Tray I
10.	0.41	0.06	2.1	0.051	0.022	2.25	0.048	Tray J
11.	0.45	0.065	-	-	-	-	-	-
12.	0.39	0.059	-	-	-	-	-	-
13.	0.405	0.06025	1.73	0.0434	0.0163	1.92	0.0406	**Average**

After stripping, spent up 2.0^{+} (second spawners) broods were managed as future broods for the next year breeding and stocked @ 15 m^{-2} (10 kg m^{-2}) maintaining WD 2.08 L sec^{-1} (0.42 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 16.4°C. Artificial fertilization was procured in the evening on Tuesday, 15 November 2011 with 108.27 ± 4.43 g zygotes female^{-1} (1299.2 g zygotes 12 females^{-1}) and 1797.09 ± 73.57 number zygotes female^{-1} (21564 number zygotes 12 females^{-1}) ensuring fertilization percentage to be 92.56 ± 0.45%.

WT ranged 8.8-21.3 (17.34 ± 1.9°C), pH 6.8-7.8 (7.31 ± 0.14), DO 7.4-10.2 (8.63 ± 0.46 mg L^{-1}), FCO 1.7-4.5 (3.4 ± 0.36 mg L^{-1}) and WD 39-92 (60.57 ± 7.17 L sec^{-1}) from June 2011 to December 2011.

Zygotes were transferred in the evening into incubation cum hatching trays put into incubation cum hatching raceways on Tuesday, 15 November 2011 maintaining WD 0.033 L sec^{-1} @ 10,000 eggs in WT 12.2°C, pH 7.6, DO 9.9 mg L^{-1}, and FCO 2.8 mg L^{-1}. Cleaning and readjustment of zygotes in incubation cum hatching trays were done four times – 1st on Tuesday, 22 November 2011; 2nd on Tuesday, 29 November 2011; and 3rd on Tuesday, 6 December 2011; and 4th on Tuesday 13 December, 2011 so as to remove dead eggs. During 4th cleaning, eyed-eggs were seen confirming hatching to occur after 1 day. Hatching of zygotes occurred on Wednesday, 14 December 2011 after 1 day of last cleaning and readjustment. Incubation period was 29 days (Figure-1 and Figure-2) in average temperature of 9.34 ± 0.17°C day^{-1} and cumulative temperature (sum total of WT of 29 days) of 269.8°C with hatching of SFs (survivability 44.03 ± 2.42%), each 1.73 ± 0.104 cm, 0.0434 ± 0.0018 g, and 0.0163 ± 0.0013 g yolk-sac (Table 1). Yolk (energy) used during the development, growth, and formation of yolk-sac (also keeping yolk inside it) due to conversion and change of

zygotes into SFs was 0.01685 g (27.97%), 0.0434 g (72.03%), and 0.0163 g (27.05% of the zygotes) respectively. Further, yolk-sac was 37.56% of sac-fries.

SFs were transferred in the evening into endogenous feeding cum hatching cages put into endogenous feeding cum hatching raceways on Wednesday, 14 December, 2011 maintaining WD 0.033 L sec^{-1} in WT 8.8°C, pH 7.8, DO 10.2 mg L^{-1}, and FCO 1.7 mg L^{-1}. Hatchability was noticed on Wednesday, 21 December 2011 after 7 days (Fig1 and 2). Hence, EFP was 7 days in WT of 8.8°C releasing FSFs (survivability 78.26 ± 2.35%) each 1.92 ± 0.097 cm and 0.0406 ± 0.0018 g (Table-1) ready for exogenous feeding. Yolk (energy) of the yolk-sac used during the development and growth due to conversion and change of SFs into FSFs was 0.0028 g (17.18%) and 0.0135 g (82.18%) respectively. Further, development of SFs into FSFs was 6.45% of sac-fries and growth 93.55% (Table-1).

DISCUSSION

Rainbow trout can be bred from September to February in India[24] and November to February in Nepal[6]. Rainbow trout breeding successfully, in this research work, in November by induced breeding resembled both[24 and 6] and also confirmed artificial method of propagation to be the successful method of breeding. Rainbow trout can be bred twice but it is bred only once during breeding season in Nepal[6 and 22] because breeding twice gives poor spawning results with mortality of broods, incubated eggs, SFs, and FSFs, and breeding only once, gives good spawning results with comparatively more survivability of broods, incubated eggs, SFs, and FSFs. According to[13], a male rainbow trout spawns at 1 yr and a female at 2 yrs; according to[22], rainbow trout breed after attaining 2 yrs; and according to[6], 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 cultivation highly depend on selection, management, age, and maturation of brood, disease surveillance, feeding, and water quality[6]. 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 [6]. Further, according to[6], 2.0^{+}broods (first spawners), should be selected based on external appearance and weight. So, most of the experimental broods (150 out of 200) were selected as future broods according to [6], 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 spent up broods total 66.392 kg, each 36.06 ± 0.28 cm long and 442.611 ± 7.979 g wt., stocked @ 15 m^{-2}, i.e., 6.6392 kg m^{-2} following [14, 6, 21, and 10] and obtaining them after 7 months (in May, 2011) as experimental broods total 82.5 kg, each 36.88 ± 0.25 cm long, 550 ± 8.939 g wt., with 100% survivability and 15.341 g growth $month^{-1}$ (for 7 months); maintained for experimental broods as future broods stocked @ 15 m^{-2}, i.e., 8.25 kg m^{-2} and obtaining them after 2 months (in July 2011) as broods total 91.35 kg, each 37.08 ± 0.25 cm long, 609 ± 4.909 g wt., with 100% survivability and 29.5 g growth $month^{-1}$ (for 2 months); maintained for broods stocked @ 15 m^{-2}, i.e., 9.135 kg m^{-2} and obtaining them after 1 month (in August, 2010) as segregated broods total 96 kg, each 38.l4 ± 0.26 cm long, 640 ± 6.557 g wt., with 100% survivability and 31 g growth $month^{-1}$ (for 1 month); maintained for segregated broods stocked @ 15 m^{-2}, i.e., 9.6 kg m^{-2} and obtaining them after 2 months (in October,

2010) as current broods total 105 kg, each 41.04 ± 0.35 cm long, 700 ± 24.988 g wt., with 100% survivability and 33 g growth month^{-1} (for 2 months); maintained for current broods stocked @ 15 m^{-2}, i.e., 10.5 kg m^{-2} and obtaining them after 1 week (in the 1st week of November 2010) as gravid broods total 106.8 kg, each 41.08 ± 0.41 cm long, 712 ± 19.404 g wt., with 100% survivability and 1 g growth day^{-1}; and maintained for all the gravid broods stocked @ 10 m^{-2}, i.e., 10.12 kg m^{-2} and obtaining them on the day of breeding as 1.0^{+}broods (first spawners) total 34.742 kg, each 39.41 ± 0.21 cm long, 694.833 ± 10.555 g wt., gave similar results like[6] because experimental trout management, future brood selection, and broods, segregated broods, current broods, and gravid broods confirmation were done accordingly. According to[11], 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[18, 3, 6, 16, 22, 28, and 10].

Yellow colour of eggs was due to the carotenoids present in the feed[23]. According to[6], eggs vary from 3-5mm in diameter. Colour and size of eggs resembled[22 and 6] respectively. According to[19], the older brood generally lays larger-sized and higher number of eggs and according to[20], a 3-4 years female lays 3000-3500 number eggs kg^{-1} body wt. Further, according to [25], there should be 20 millions spermatozoa ml^{-1} milt. According to[27] reproductive efficiency in rainbow trout was due to age dependent changes hence, fertilization percentage, eggs survival, and larvae survival was more with larger broods and less with smaller. Less number of eggs (2781 eggs in number) each 4.05 mm in diameter and similar numbers of spermatozoa (20 millions ml^{-1}milt) in the present work was due to the age of broods being 2 yrs and 10 months.

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 SFs; and during hatchability to ensure more survival of SFs releasing 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[6], one male can supply enough milt for the fertilization of eggs of two females. Here, fertilization was 2 : 1: : eggs : milt just like reported by[6].

Rainbow trout mostly requires glacier water or clean cold spring water for its successful breeding[6 and 10]. Water resource supplying water in the raceways situated at the ALT of 1550 msl, in the present investigation, was permanent, dependable, and from perennial spring-fed stream. The prerequisite for rainbow trout cultivation is adequate volume of coldwater 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 [22]. It is known that rainbow trout require WT 0-25°C[28] and 8.4-21.5°C[7] but according to[30], it grows well in WT 10-18°C, however, according to[1], its best growth in Nepal occurs in WT 16-18°C. According to [22], 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 8.8-12.2°C.

The preferable pH for rainbow trout is 6.5-8.0 with optimum value 7.0-7.5[22] and 6.7-7.9[7] for semi-intensive cultivation because at higher pH levels, relatively low levels ammonia (NH_3) can be dangerously toxic[12 and 26]. According to[15], rainbow trout require DO above 7.0 mg L^{-1}; according to[22], it requires DO more than 7.0 mg L^{-1}; according to[6], its brood requires cold, clean and high DO containing water of 7.0-7.5 mg L^{-1} for normal trout cultivation and 10-11 mg L^{-1} for intensive cultivation for proper ripening of gonads and successful hatching of SFs and FSFs; and according to[7], DO at the level of 7.2-10.5 mg L^{-1} is suitable for rainbow trout cultivation

because according to [2], 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}.[17] So, induced breeding under semi-intensive cultivation was done in the present work because pH was 6.8-7.8 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 mentioned above.

As a rule of thumb, hatching of eyed-eggs into SFs 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 [22]. In the present work, the cumulative WT was 269.8°C in the hatching period of 29 days. SFs growing period of rainbow trout is from March to April when egg is surrounded by yolk-sac (indicating endogenous feeding period) nourishing the developing egg nucleus[29]. The present investigation showed that SFs growing period in Nepal was from November to December because of the presence of suitable WT during the period.

SFs having yolk-sac obtained from well-fed 3 years matured female and male brood show more survival, growth, activeness, and more yolk-size of SFs than those not well fed but of the same age and also to those of 2 years of age. Similarly, SFs obtained from well-fed 2 years matured female and male brood show more survival, growth, activeness, and more yolk-size of SFs than those not well fed but of 3 years age[19]. Water quality, brood maintenance, and artificial feed play an important role in achieving higher hatchability[6]. Because hatchability was survivability, therefore, it was less being 44.03 ± 2.42% probably due to the age and size of the brood.

According to[14], 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[3] and 20-30 days depending on water temperature[22]. 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[6]. High WT during spawning season claimed deformed SFs [5]. No deformed SF was found as the WT was quite suitable. The incubation period was 29 days in WT of 9.34 ± 0.17°C day^{-1} in the present work.

The yolk-sac got absorbed within 5-7 days[19], 7-18 days[6], and 5 days[10]. The yolk-sac of yolk-sac fry of rainbow trout weighing 0.08 g was found absorbed in 2 weeks[22]. Endogenous feeding period depends on WT[8]. It was 7 days in WT of 8.8°C the present investigation.

After hatching, SFs are carefully removed from the trays into the freely-hanging hatching cages where running water is maintained by protecting them from bright light. WD in the freely-hanging hatching cages was @ 0.3-0.5 L sec^{-1} for 10,000 SFs. Hatchability was low in Godawari and Trishuli than in Kakani and Rasuwa. On the first day, immediately after hatching, SFs are 1.3-1.8 cm in length and 40-50 mg in weight [22]. A larva, also known as SF, measures about 1.3-1.8 cm in length and 50-80mg in weight. Out of the total weight of SF, yolk-sac constitutes about 50-60% weight [22]. In the present work, zygotes which were converted into SFs were 1.73 ± 0.0104 cm in length and 0.0434 ± 0.0018 g in wt. with 0.0163 ± 0.0013 g wt. of yolk-sac. So, conversion and change of yolk of zygotes to SFs was 27.97% for development, 72.03% for growth, and 27.05% for yolk-sac which was 37.56% of SFs. Similarly, in the present work, SFs which were converted into FSFs were 1.92 cm in length and 0.0406 g wt. Therefore, conversion and change of yolk of yolk-sac of SFs to FSFs was 17.18% for development and 82.18% for growth which respectively was 6.45% of SFs for development and 93.55% of SFs for growth. Hence, conversion and change of zygotes to SFs 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 SFs 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 SFs along with proper shelter. However, SFs weighing 0.08 g become FSFs in 2 weeks[22]. In the present work, total incubation period from zygotes to FSFs was 36 days which was due to the moderate WT of 8.8-12.2°C. Results confirmed the endogenous feeding period of 7 days in WT of 8.8°C in the present investigation.

CONCLUSION

Results of 2 years and 10 months old rainbow trout broods confirmed the total incubation period (incubation period + endogenous feeding period) of zygotes to FSFs being 36 days from 15 November to 21 December 2011 depending on WT in the WT of 13.1°C. Hence, conversion and change of the yolk of zygotes into SFs for development, growth, and formation of yolk-sac and further, yolk of the yolk-sac of SFs into FSFs for development and growth were comparatively less because of the small age and size of the broods. Further, the incubation period of zygotes to SFs was 29 days in WT of 9.34 ± 0.17°C day^{-1} and cumulative WT of 269.8°C with 44.03 ± 2.42% survivability releasing SFs each 1.73 ± 0.104 cm, 0.0434 ± 0.0018 g, with 0.0163 ± 0.0013 g yolk-sac. Furthermore, the endogenous feeding period of SFs to FSFs was 7 days in WT of 8.8°C with 78.26 ± 2.35% survivability releasing the FSFs each 1.92 ± 0.097 cm and 0.0406 ± 0.0018 g which were ready for exogenous feeding period.

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