International Journal of Fisheries and Aquatic Studies 2025; 13(1): 151-155

E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(1): 151-155

www.fisheriesjournal.com
Received: 24-11-2024
Accepted: 27-12-2024

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Optimizing rainbow trout *(Oncorhynchus mykiss)* Breeding: An assessment of age and performance relationship in nepalese hatcheries

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DOI: https://doi.org/10.22271/fish.2025.v13.i1b.3031

Abstract

The study was conducted to determine the effect of female age on the breeding performance of Rainbow trout at Rainbow Trout Fishery Research Station, Dhunche, from Poush 2079 to Chaitra 2079. The study was carried out in a Completely Randomized Design (CRD) with 5 replications, and each replication consists of 3 treatments of different age groups, *viz*., 1+ female, 2+ female, and 3+ female. Breeding performance parameters, *viz*., absolute fecundity, relative fecundity, egg diameter, fertilization rate, hatching rate, egg incubation period, full yolk sac absorption days, alevin to swimmer stage survivability weight, length of first feeding fry, and their production cost, were economically analyzed. Among the treatments, 3+ females exhibited significantly higher absolute fecundity (2165±90.800), egg diameter (4.20±0.174 mm), egg hatching rate (69.48±2.401%), alevin to swimmer stage survivability (97.60±0.602), weight of the first feeding fry (132.600±4.790 mg), and length of the feeding fry (2.488±0.054 cm). Similarly, 1+ females recorded the significantly highest relative fecundity (3207±52.600), egg incubation period (45.60±1.445), and full yolk sac absorption days (74.80±1.433). In terms of economic analysis, 3+ females reported the highest net profit, while the best BC ratio was observed among 2+ females.

Keywords: Rainbow Trout, breeding performance, benefit/cost analysis, breeding age

Introduction

Rainbow trout (*Oncorhynchus mykiss),* being a carnivorous cold-water fish, is probably one of the oldest fish in culture after common carp. Its artificial breeding was first done in North America around 1872 A.D. (Gall & Crandell, 1992) [11]. In Nepal, 50,000 rainbow trout-eyed eggs were brought from Miyazaki Prefecture of Japan in 1988 A.D. and hatched in a fish farm, Godawari (now the National Fishery Research Center). Then, its breeding and experimentation on cultivation were initiated around 1990 and 1993 A.D., respectively (Rai *et al.*, 2008; Swar, 2008) [19, 27]. Now in Nepal, rainbow trout farming covers 3.1 hectares of water surface area and contributes about 320 metric tons to the total fish production of 83,544 metric tons. The rainbow trout farming growth rate averages 1.59 percent (CFPCC, 2019) [8].

The water temperature ranges from 9 to 14 °C and is suitable for maintaining broods and egg incubation (Rai *et al.*, 2008) [19]. Rainbow trout exhibit a reproductive cycle consisting of one spawning and spermiation period per year, usually in the winter (Peter & Crim, 1979) [16]. Males and females aged 22 to 24 months attain breeding age in Nepal between the months of Mangsir and Falgun (Rai *et al.*, 2008) [19]. The careful selection of high-quality brood fish is one of the most important aspects of improving hatchability and lowering mortality. To meet the national yearly demand for about 20 lakh eggs, the trout industry needs to study and boost the reproductive potential of existing broodstocks (RTFRS, 2019) [22]. A controversy exists in the literature concerning the relationship between brood age and egg survivability in Salmonids. Some authors conclude that small eggs from young brood have the lowest survival rates (Small, 1979; Pitman 1979) [25, 17], while others support the idea that survival is independent of egg size (Glebe *et al.*, 1979; Thorpe *et al.*, 1984; Bromage & Cumaranatunga, 1988) [12, 28, 7]. In this study, we evaluated the ideal age groups for breeding efficiency by assessing metrics such as fecundity, fertilization, hatching rate, and the survivability of alveins. We also compared the cost-benefits ratios associated with producing fries from broods of various ages.

Materials and Methods

The expeiment was carried out under controlled hatchery conditions at Rainbow Trout Fishery Research Station (RTFRS), Dhunche, Rasuwa (28°6'37" N, 85°18'20" E), Bagmati Province of Nepal, located at an altitude of 1,850 meters above sea level. The study was conducted from the third week of Poush 2079 to the second week of Chaitra 2079 (January 2022 to March 2022). The research was carried out in a Completely Randomized Design (CRD) with three treatments, i.e., first 1+ female, 2+ female, and 3+ female, replicated five times. Different ages of males and females were determined based on their body weight and length, selected from graded brood fish ponds. During the culture period of a few weeks, brood fish were fed trout feed (commercial extruded trout feed) of crude protein (34.9%) at a rate of 1 percent of their live weight before spawning. The state of ripeness was examined twice a week.

The gravid broods were selected on the 17th of poush and brought into the hatchery. In a plastic bowl, eggs were collected from each female by hand stripping, and the weight of each female and egg mass was measured with a digital balance. Later, the eggs were counted separately by weighing 1gm egg, and a digital Vernier caliper was used to measure the diameter of 10 eggs. After that, the milt of males was stripped and poured directly into the egg-containing bowl, maintaining a sex ratio of 2:1 (Male: Female), stirred gently by a feather, and waited for a minute for fertilization. Then, about a 0.9 percent salt solution was carefully poured from the side of the bowl to remove dirt and clean the fertilized eggs. Then the yellowish eggs were washed continuously until the water became transparent. Finally, the water-hardened eggs of different treatments were poured into 15 trays (locally made 33×34 cm in size). And three trays were tied in stakes together to put into the fiber atkins, maintaining five replications. A Continuous and uniform water flow was maintained at a rate of 2 L/min. After 26 days of incubation, 1,000 eyed-eggs of each treatment were replicated in Chinese atkins (0.40 m × 0.175 m × 0.09 m) maintaining a water flow rate of 6 L/min till the free-swimming stage. During the ongoing process of hatching, their average population and hatching dates were noted. Dead and deformed alevins were removed daily until they reached the swimming stage. Atkins as well as the water inlet pathway were cleaned through siphoning at succeeding 2 day intervals. After full absorption of the yolk sac, the average length and weight of swimmers or early fries from each experimental unit were recorded and then treated with 2% NaCl.

Data entry and tabulation were done in MS-excel. One-way ANOVA was used to determine the significant effect of treatments. Least significant difference (LSD) test was conducted by using Genstat statistical analysis package at a 5% level of significance. DMRT was used to compare mean values. A simple economic analysis was conducted to determine economic returns from each treatment, and the returns from each treatment were compared to identify suitable brood ages in terms of economic return.

Total costs= variable costs+ fixed costs

Gross revenure= the total amount of production(kg) multiplied by their respective market prices

Net Profit= Gross revenue-total costs

Benefit-cost ratio= Gross revenue divided by total production costs

Gross margin (NRs)= Gross revenue (NRs)- total variable costs(NRs)

Results and Discussion

Treatment effect on body weight, length and fecundity

The present study demonstrated the significant effects of different treatments on body weight, length, absolute fecundity, and relative fecundity (Table 1). As age increased, we observed a correlating rise in body weight and length. The highest body weight and length were recorded by 3+ females, which were 0.778±0.028 kg and 41.140±1.051 cm respectively. This might be attributed to older fish typically being larger (Thorpe *et al*., 1984) [28]. Rahbar *et al*. (2011) [18] in the Caspian brown trout recorded similar results, i.e., increased body weight and length with age.

In terms of absolute fecundity, 3+ females performed the best, with the highest absolute fecundity (2165±90.800) while the lowest absolute fecundity (1474±90.800) was recorded in 1+ females. Fish fecundity is known to increase with the age of brooders (Reznick *et al*. 2002) [20]. Further, due to the high weight of 3+ females, it might have recorded the highest absolute fecundity because there is a direct relationship between body weight and absolute fecundity (Estay *et al*., 1994) [10]. Among different treatments, the highest relative fecundity (3207±52.600) was recorded in 1+ females. This might be because relative fecundity has been expected to decline with age (Siraj *et al*., 1983; Ridha & Cruz, 1989) [24, 21]. Similarly, young fish have generally higher relative fecundities (Bromage *et al*., 1992; Bobe & Labbe, 2010) [7, 4]. Further, in our study, egg size, i.e., egg diameter, increased with age (Table 2), which might have caused decreased relative fecundity with age because relative fecundity decreases when egg size increases, either with female size (Lobon-Cervia *et al*., 1997) [14] or with female age (Belding, 1940; Baum & Meister, 1971) [3, 2]. Similar findings were also documented in Caspian brown trout (Rahbar *et al*., 2011) [18] and Nile tilapia (Valentin *et al*. 2015) [29].

Table 1: Weight and length of different female age with their respective absolute and relative fecundity at RTFRS, Rasuwa, Nepal

TMT	Female weight (kg)	Female Length (cm)	Absolute Fecundity	Relative Fecundity
1+	0.460c	34.140c	1474c	3207a
2+	0.622b	36.500b	1859b	2988b
3+	0.778a	41.140a	2165a	2782c
SED (±)	0.028	1.051	90.800	52.600
LSD (5%)	0.061	2.290	197.800	114.700
CV %	7.200	4.500	7.800	2.800
F test	***	***	***	***

Treatment effect on egg diameter, fertilization rate and hatching rate

Different Different treatments showed significant effects on egg diameter and hatching rate, while no significant effect was recorded on fertilization rate (Table 2). The highest egg diameter (4.20±0.174 mm) was recorded in 3+ females. 1+ female and 2+ female did not show any significant differences in egg diameter. The increase in egg diameter with age may be due to the fact that as the size and age of brood fish increase, the egg size also increases (Bromage *et al.* 1992) [7]. Nomura (1963) [15] and Springate *et al.* (1984) [26] showed a positive relationship between egg size and fish size in rainbow trout. Similar results were recorded by Kurtoglu. Likewise, Caspian brown trout (Rahbar *et al.*, 2011) [8] and common carp (Aliniya, 2013) [1], showed increased egg size with the increase in the age of females. An increase in fertilization rate with age might be due to uniform maturation. Similarly, Bozkurt *et al.* (2006) [5] observed a positive relationship between an increase in egg size and the success of fertilization rate, and our results also recorded an increased fertilization rate with the increase in egg size. Chandra *et al.* (2018) [9] recorded an increased fertilization rate with the increase in weight of female rainbow trout.

The egg-hatching rate increased with age. Among different treatments, the highest egg hatching rate (69.48±2.401%) was obtained in 3+ females. This finding is supported by Pitman (1979) [17], who found a higher hatching rate in the older female.

Table 2: Effect of female age on egg size, fertilization rate and hatching rate at RTFRS, Rasuwa, Nepal

TMT	Egg diameter (mm)	Fertilization rate (%)	Hatching rate (%)
1+	3.520b	99.690a	56.550 c
2+	3.720b	99.920a	63.330 b
3+	4.200a	99.960a	69.480 a
SED (±)	0.174	0.199	2.401
LSD (5%)	0.380	0.434	5.232
CV %	7.200	0.300	6.000
F test	**	NS	***

Treatment effect on egg incubation period, full yolk absorption days and alevin to swimmer stage survivability

Different treatments showed a significant effect on egg incubation period, full yolk absorption days, and alevin to swimmer stage survivability. The egg incubation period decreased with the increase in the age of females. In our results, the highest egg diameter was in 3+ females (Table 2), so 3+ females might have recorded the lowest egg incubation period. This could be due to egg size inversely influencing the incubation period, i.e., a larger egg will result in a shorter incubation period (Savadkouhi & Khara, 2017) [23].

Full yolk sac absorption days decreased with the age of the female. The highest full yolk sac absorption days (74.80±1.433) were recorded in 1+ females, while the lowest full yolk sac absorption days (68.80±1.433) were recorded in 3+ females. Similarly, 2+ females and 3+ females did not show any significant differences in full yolk sac absorption days. The alevin to swimmer stage survivability increased with the age of the female. Among different treatments, the highest alevin to swimmer stage survivability (97.60±0.602) was recorded in the 3+ females, while the lowest survivability (90.39±0.602) was observed in the 1+ female.

Table 3: Effect of female age on egg incubation period, full yolk absorption days and alevin to swimmer stage survivability at RTFRS, Rasuwa, Nepal

TMT	Egg incubation period	Full yolk sac absorption days	Alevin to swimmer stage survivability (%)
1	45.600a	74.800a	90.390 c
2	43.200b	70.000b	95.570 b
3	42.400b	68.800b	97.600 a
SED (±)	0.663	1.433	0.602
LSD (5%)	1.445	3.122	1.311
CV %	2.400	3.200	1.000
F test	***	**	***

Treatment effect on First feeding fry weight and length

Different treatments showed a significant effect on the weight of the first feeding fry and the length of the first feeding fry (Table 4). The weight and length of the first feeding fry were found to increase with the increase in the age of females. The high weight and length of the first feeding fry might be due to the large egg diameter.

Table 4: Effect of female age on first feeding fry weight and length at RTFRS, Rasuwa, Nepal

TMT	Weight of first feeding fry (mg)	Length of first feeding fry (cm)
1	86.200c	2.192c
2	121.600b	2.342b
3	132.600a	2.488a
SED (±)	4.790	0.054
LSD (5%)	10.440	0.118
CV %	6.700	3.700
F test	***	***

Bio-economic analysis

The The highest total fixed cost in 3+ was due to the high price of 3+ broodstock due to its large weight. The highest variable price in 3+ was due to more consumption of feed by the larger broods and more fries. The highest net profit from 3+ was due to high relative fecundity and high hatchability. The highest BC ratio was found on 2+, which was 1.64 due to a lower production cost than 3+, while the lowest BC ratio was found on 1+, which was 1.54. The 3+ did not record the highest BC ratio due to its high production cost, and the 1+ did not record the highest BC ratio due to low income from fries due to low fecundity.

Table 5: Fixed cost for rearing broods

TMT	Fixed cost			
	Broodstock cost (NRs)	**Labour wage (NRs)**	**Rent (NRs)**	**Total (NRs)**
1+	5290	5922	5750	16962
2+	7153	5922	5750	18825
3+	8947	5922	5750	20591

Table 6: Variable cost for rearing broods

TMT	Variable cost		
	Brood feed (NRs)	**Fry feed (NRs)**	**Total (NRs)**
1+	242	132	374
2+	300	282	582
3+	375	401	776

Table 7: Net profit and BC ratio from different age group

TMT	Net profit and BC ratio				
	No. of sold fries	**Income from fries (NRs)**	**Production cost (NRs)**	**Net profit (NRs)**	**BC ratio**
1+	3273	26184	16962	9222	1.54
2+	3858	30864	18825	12039	1.64
3+	4128	33024	20619	12405	1.60

Conclusion

The present study examined the influence of age on various aspects of brood fish growth and subsequent productivity, incorporating parameters such as body weight, length, fecundity, egg diameter, fertilization rate, hatching rate, egg incubation period, full yolk sac absorption days, alevin to swimmer stage survivability, and first feeding fry weight and length. We reported 3+ females demonstrating the highest body weight (0.778±0.028 kg), length (41.140±1.051 cm), and absolute fecundity (2165±90.800). Relative fecundity, however, was highest in 1+ females (3207±52.600). The study also shows that egg size increases with age. However, age did not significantly impact the fertilization rate. In terms of hatching, 3+ females had a higher hatching rate (69.48±2.401%). The results also demonstrated a decrease in the egg incubation period and full yolk sac absorption days with increasing female age. In addition, 3+ recorded the highest alevin to swimmer stage survivability (97.60±0.602%), the highest weight of the first feeding fry (132.600±4.790 mg), and the length of the feeding fry (2.488±0.054 cm). Furthermore, the bio-economic analysis suggested that while 3+ females resulted in the highest net profit, the best BC ratio was found with 2+ females. Therefore, despite the good performance of the 3+ females from a biological perspective, the 2+ females were more economically efficient. Hence, it is suggested to use 2+ female brood fish to make the hatchery business profitable and prosperous.

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