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Research Article

Artificial breeding success, incubation period, and fecundity of rainbow trout, *Oncorhynchus mykiss* (Kamloops Strain), in Jaghour trout hatchery, Chitral

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Abstract

Rainbow trout (*Oncorhynchus mykiss*; Kamloops strain), were introduced to Khyber Pakhtunkhwa in 1928 and are now successfully farming in the Malakand and Hazara divisions due to the region's favorable cold-water conditions. Our study investigated the artificial breeding success, incubation period, and fecundity of rainbow trout at Jaghour trout hatchery in the district Chitral. A total of 4,311 eggs were harvested from four females with a combined body weight of 1.264 kg. The eggs were fertilized and incubated in spring water at temperatures ranging from 12.6 to 13.3°C. The survival and mortality rates from fertilization to hatching were approximately 79% and 21%, respectively. The eyed stage commenced at around 155±05 degree days (D°), with hatching occurring at approximately 305±05 D°. The incubation period spanned from 305±05 to 349±05 D°. Furthermore, the study explores female fecundity, revealing a range of 1.87 to 3.42 eggs per gram of body weight (n=15). The size of green ova increased by 1.02% to 8.60% during water hardening at 9°C for 1.5 h (n=46). Our study provides valuable insights into the incubation period of Rainbow trout, highlighting the survival and mortality rates of fertilized eggs in spring water. This information can assist local hatchery managers in estimating seed production from known brood stock.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is an important freshwater fish species widely distributed in the Northern Hemisphere, valued for both aquaculture and recreational fishing. The Kamloops strain, known for its rapid growth and adaptability, is a popular choice for aquaculture in the region (Fornshell, 2002; Singh *et al*., 2016). Studies have shown that different strains of rainbow trout, such as the Case Western strain, exhibit varying levels of thermal tolerance and growth potential at elevated temperatures (Hartman and Porto, 2014; Li *et al*., 2024). Distribution of rainbow trout, including the Kamloops strain, is widespread in aquaculture. According to the United Nations Food and Agriculture Organization (FAO), rainbow trout production exceeded 700,000 tons in 2009, highlighting the global significance of rainbow trout aquaculture (Pauly and Zeller, 2017; Vasdravanidis *et al*., 2022). Effective aquaculture practices for rainbow trout require careful consideration of factors such as water temperature, oxygen availability, feed quality, and environmental conditions. Rainbow trout are classified as cold-water fish with an optimal environmental temperature of 15°C (59°F) (Savić and Mikavica, 2022; Sedgwick, 1995).

Trout production at a commercial level necessitates a thorough understanding of their biological and ecological needs. Proper training and capacity building of human resources and progressive fish farmers in fish breeding, feeding, hatchery management, and pond management are prerequisites for commercial-scale trout production (Hassan *et al*., 2007; Savić and Mikavica, 2021). Maintaining suitable environmental conditions is crucial for optimizing growth rates and overall health in aquaculture settings (Klontz, 1991; Savić and Mikavica, 2021, 2022). In well-maintained and deep waters, rainbow trout can withstand temperatures between 20°C and 22°C, and momentarily as high as 24°C. They are more resistant to certain diseases, notably furunculosis. Rainbow trout develop rapidly, have a short incubation period, and exhibit fast growth. They are less carnivorous and cause minimal predation on younger fish. The spawning period for rainbow trout typically ranges from January to May, but it can vary depending on the strain, regional climate, water conditions, and health of the spawners (Huet, 1986; Kocaman *et al*., 2009; Sarma *et al*., 2018).

The spawning period can be advanced through selective breeding, the use of artificially controlled light, and the injection of pituitary hormones (Leitritz and Lewis 1976; Hoitsy *et al*., 2012).

In Pakistan, rainbow trout are cultured in the Malakand and Hazara divisions, Kurram district of Khyber Pakhtunkhwa, District Murree of Punjab, District Quetta in Balochistan, and Gilgit-Baltistan due to their compatibility with the environment and the availability of high-quality water. The increase in productivity of the Kamloops strain in Khyber Pakhtunkhwa dates back to 1984-85 and 1986 when 500,000 eggs were imported from the USA under the Pakistan Aquaculture Development project. This importation significantly contributed to establishing sustainable populations in rivers, streams, and lakes of the northern districts (Yaqoob, 2002).

Artificial breeding is essential for the commercial propagation of trout fish. In Pakistan, the focus is on producing juvenile trout for release into natural waters to enhance wild populations rather than large-scale commercial farming for market consumption (Yaqoob, 2002; Laghari, 2018).

This study aims to assess the incubation period of rainbow trout and highlight the survival and mortality rates of fertilized eggs using spring water at Jaghour Trout Hatchery (JTH). By estimating relative and absolute fecundity, hatchery managers can better predict their seed production from known brood stock in their production units.

Materials and methods

Study Area

Chitral is the largest and northernmost administrative district of Khyber Pakhtunkhwa, covering an area of 14,850 km² and situated approximately 365 km from Peshawar, the provincial capital of KP. The district is rich in torrential cold water streams, high-altitude lakes, crystal-clear perennial cold water springs, snowcapped peaks of the Hindukush Mountainous Range, and is renowned for its unique ancient Kalasha culture. Due to its remote location in rugged mountainous terrain, job opportunities and resources for income generation are scarce, leading to prevalent poverty among the local communities. The locals rely on limited resources such as livestock, agriculture, timber, and government services for earning a livelihood. With proper utilization of natural water resources and support through technical and financial assistance, initiatives like trout culture and angling-based tourism in farms and natural water bodies could offer alternative sources of income for the local population.

Jaghour Trout Hatchery (JTH), located at N 35˚49’07.7”, E 71˚46’21.5”, with an altitude of 1460m, is a public sector hatchery situated approximately four kilometers from Chitral town on the N-45 Dir-Chitral road in Shouth village, Jaghour Chitral **(**Fig. 1**)**. The hatchery benefits from both stream (Jaghour Gol) and spring waters. Spring water is predominantly used for incubating eggs, rearing alevins, and fry due to its optimal temperature for spawning and growth, lack of pollutants, perennial availability, and low turbidity. A combination of stream and spring water is utilized for rearing fingerlings, yearlings, marketable fish, and brood stock. In the study area at JTH, the breeding season for Rainbow trout (Kamloops strain) typically commences in late October or early November and extends until February.

Data collection

Selection and stripping of spawners

After an initial examination of approximately 800 brood stock at JTH, 60 sexually mature female and 30 male brooders were selected and housed separately in a fingerling raceway. The raceway measured 20 feet (ft) in length, 4 ft in width, and 4 ft in depth, with a water level of 2 ft. As a standard practice, the brooders are typically fasted for 48 to 96 h (2-4 days) to prevent contamination of milt and eggs with fecal matter and reduce stress during the stripping process. In this particular study, the broodstock was fasted for 72 h before stripping. To ensure gametes quality, firm and transparent eggs were collected from female spawners as limp and translucent eggs are considered of poor quality. Similarly, white and milky milt was utilized as watery and curdled milt is of poor quality. During the study, 4 females and 2 males were stripped in the hatching room, with cumulative weights of 1.264 kg for the females and 0.586 kg for the males. The spawners were stripped without using anesthesia.

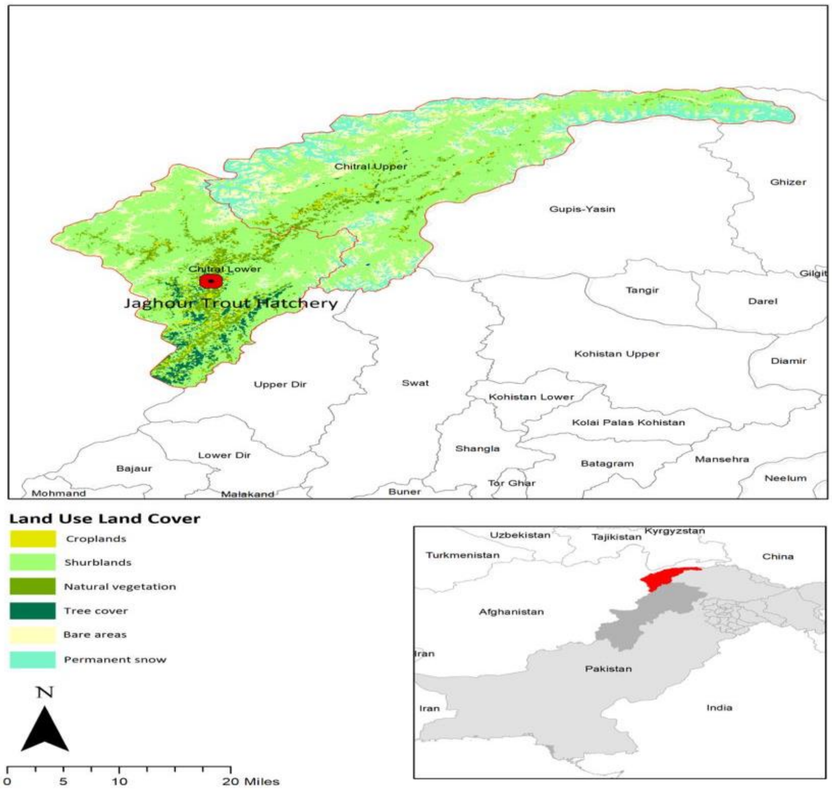

Figure 1: Study Area Map.

The stripping process involved initially extracting gametes from 2 females followed by one male in a receptacle, gently mixing them with water using duck feathers. Subsequently, the remaining 2 females and one male were stripped into the same receptacle and mixed gently. Likewise, rough handling was avoided during stripping as rough handling can damage fragile eggs and rupture blood vessels, contaminating the eggs with blood. After a resting period of 2 min for the mixed gametes, a small amount of spring water (average temperature 13.6 °C) was gently added to activate any inactive sperm and harden the eggs. The fertilized eggs were left undisturbed for 15 min and then washed five times with spring water to remove excess sperm.

Transfer of fertilized eggs to incubator

After 20 min of stripping and fertilization, the eggs were carefully transferred to an incubation tray within a vertical flow incubator equipped with eight trays (dimensions: 40 cm L x 32 cm W x 5 cm

D). A feather was used to distribute the eggs evenly within the tray.

Water supply to the incubator

The water of the incubators was supplied via a conduit having a 1.5 inch diameter at a flow rate from 10 to 15 liters/min. Water quantity was determined by the volumetric method. Water from the conduit was collected in a container. Time was noted with a stopwatch and water volume was measured with a graduated cylinder.

Estimation of fertilized eggs

To estimate the total number of eggs, a 5 cm x 5 cm sampling frame made from local mesh was used. Eggs from the incubation tray (Five h post-fertilization) were spread on the mesh using a feather, and the number of eggs was manually counted. This process was repeated three times, and the average egg count was used for estimation. The total number of eggs in the incubation tray was then estimated by considering the average number of eggs per sample and the tray's dimensions.

Estimation of incubation period and eyeing stage

In order to estimate the incubation period of Rainbow trout eggs, water temperature within the incubator tray having fertilized eggs was recorded at 8:00 AM, 12:00 PM, and 4:00 PM using a digital thermometer (CE WT-1, TBT Scitech, China) on a daily basis until hatching occurred. The average water temperature for the duration from fertilized eggs to the hatching was calculated and multiplied by the number of days resulting in the incubation period. Additionally, the eyed stage/eyed-ova was counted for the Rainbow trout.

Removal of dead eggs

Dead eggs were identified by their white, opaque appearance **(**Fig. 2**)** and removed daily using a dedicated hose (approximately 4 ft long with a 7mm internal diameter). Care was taken to minimize disturbance to healthy eggs during this process.

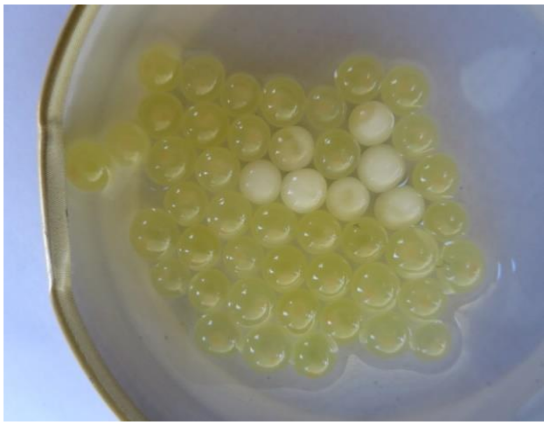

Figure 2: Live and dead fertilized ova of rainbow trout.

Estimation of absolute and relative fecundity

In order to investigate absolute and relative fecundity, the 15 trial specimens of Rainbow trout were stripped first and then weighed with the help of a digital balance. The trial specimens were kept separate for three days as they retained some eggs during the first stripping. After three days the trial specimens were re-stripped and the number of collected ova was added to the previous data. Absolute fecundity was calculated by counting the number of total eggs obtained from the trial specimen. Relative fecundity was determined by dividing absolute fecundity with the total body weight of trial specimens in grams.

Assessment of green and water-hardened ova diameter

To assess green ova diameter and volume increase, 46 numbers of green ova were collected from female spawners other than those used in the study for survival and mortality rates and incubation period. The diameter of each green ovum was measured using a micrometer mounted on a stereomicroscope. Subsequently, each green ovum was placed in a petri dish containing water at an ambient temperature of 9.0°C for 1.5 h. After absorption of water, the diameter of the water-hardened ova was then measured using the micrometer, and the percent increase in size was calculated before and after water absorption. All methods used in this study were performed in accordance with the relevant guidelines and regulations.

Statistical analysis

Statistical analysis was conducted using SPSS 20 (20 IBM, USA) software, including minimum, maximum, averages, and correlation analysis, using the Chi-Square test (χ^2) (Aqib *et al*., 2017), with a *p*-value less than 5% ($p<0.05$) considered statistically significant.

Results

Temperature-dependent, incubation dynamics, and mortality rates of rainbow trout eggs in chitral region

The present study revealed that the eyed stage started appearing at 155±05 D°, and hatching began at about 305±05 D°. The incubation period lasted from 305±05 to 349±05 D. The survival/spawning rate and mortality rate of the eggs were 3395 (79%) and 916 (21%), respectively. Before the eyed stage, 544 eggs died (59% of total mortality). After the eyed stage, 372 eggs died (41% of total mortality). The highest daily mortalities were recorded on days 9, 8, and 10 after fertilization, with 222, 127, and 65 dead eggs collected, respectively **(**Fig. 3).

Absolute and Relative fecundity of rainbow trout in Jaghour Trout Hatchery, Chitral

Our study explored the relationship between absolute fecundity (total egg number), relative fecundity (eggs per unit body weight), and body weight in Rainbow Trout (*Oncorhynchus mykiss*) at the Jaghour Trout Hatchery (JTH), Chitral. Female Kamloops strain rainbow trout at Jaghour Trout Hatchery, Chitral exhibited a range of absolute fecundity from 806 to 1,980 eggs, with an average of 1,228.13 eggs. Relative fecundity varied between 1.87 and 3.42 eggs per gram of body weight, averaging 2.67 eggs per gram (Fig. 4). This variability suggests that egg quantity is influenced not only by the female's body weight but also by a wide range of intrinsic factors i. e age (Leitritz and Lewis 1976; Rahbar *et al*, 2011), genetic makeup and extrinsic factors i. e feed quality and quantity, daily feeding ratio, water temperature, and management practices (Okumus, 2002). A statistically significant difference (p=0.0011) was observed between absolute and relative fecundity, highlighting the importance of considering both measures when evaluating fish fecundity.

The body weight of the fish ranged from 300 gm to 876 gm, with an average of 462.67 g and a standard deviation of 127.73 g, indicating some variability. Absolute fecundity ranged from 806 to 1980 eggs, with a standard deviation of 311.66, while relative fecundity had a standard deviation of 0.43.

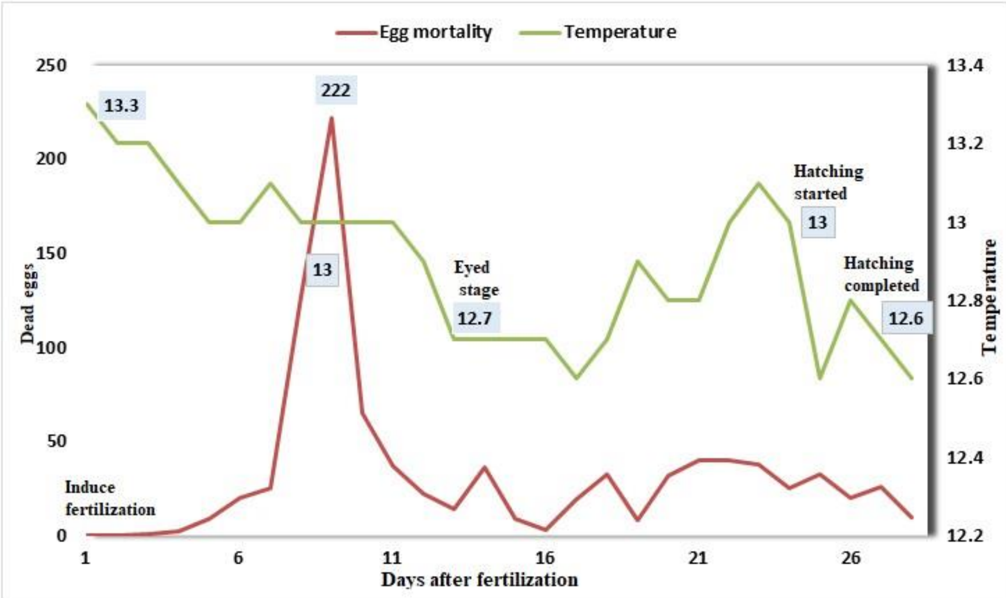

Figure 3: Temperature-Dependent Incubation Dynamics and Mortality Rates of Rainbow Trout Eggs in Chitral Region. On December 28, 2016 (Day 1) at 1:20 PM, four females and two males were stripped and artificially fertilized. Eggs were checked at 9:40 AM. Black spots began appearing but were inconspicuous. Rechecked at 3:17 PM, black spots were comparatively more conspicuous. The eyed stage began appearing at about 155±05 D° (Day 13). Four sac fry/alevins were observed at 9:00 AM on Day 24. Hatching started at about 305±05 D° (Day 24), and hatching was complete on Day 28.

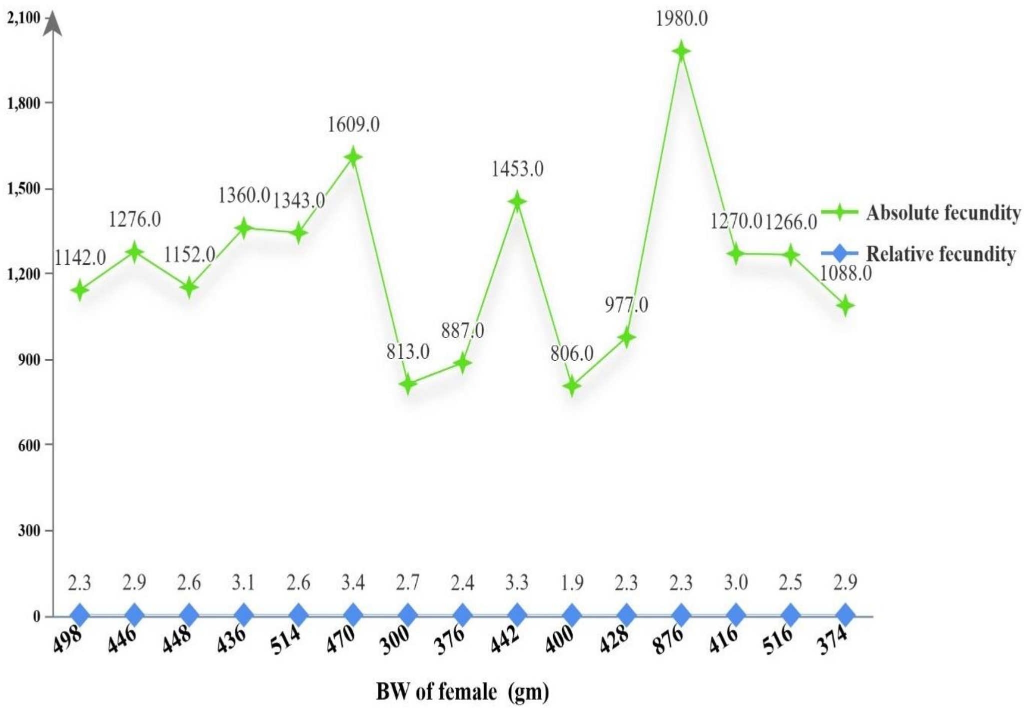

Figure 4: Relationship between Absolute Fecundity, Relative Fecundity and Body Weight in Female Kamloops Rainbow Trout Absolute fecundity ranged from 806 to 1,980 eggs (average 1,228.13 eggs). Relative fecundity ranged from 1.87 to 3.42 eggs/g (average 2.67 eggs/g). Body weight ranged from 300 to 876 g (average 462.67 g). A statistically significant positive correlation (r = 0.78; p = 0.015) was found between body weight and absolute fecundity.

A strong positive correlation (r=0.78; *p*=0.015) was found between body weight and absolute fecundity, supported by regression analysis. The regression equation, Absolute Fecundity = 221.56+2.18*BW, indicates that for every 1 gram increase in body weight, there is a corresponding increase of approximately 2.18 eggs in absolute fecundity. The R-squared value of 0.85 suggests that 85% of the variation in absolute fecundity can be explained by body weight, highlighting a statistically significant positive relationship between the two variables.

Egg size variation in response to water uptake

We compared the diameter of green ova (eggs before water hardening) to the diameter of water-hardened ova. The percentage increase in ova diameter after water hardening ranged from 1.18% to 9.52%, with an average increase of 6.25% (standard deviation: 2.13%). The majority of ova diameter increases fall within a cluster around the mean value **(**Fig. 5**).** There is a statistically significant increase in Green Ova diameter compared to Water-hardened Ova diameter, as indicated by a *p*-value of 0.0001.

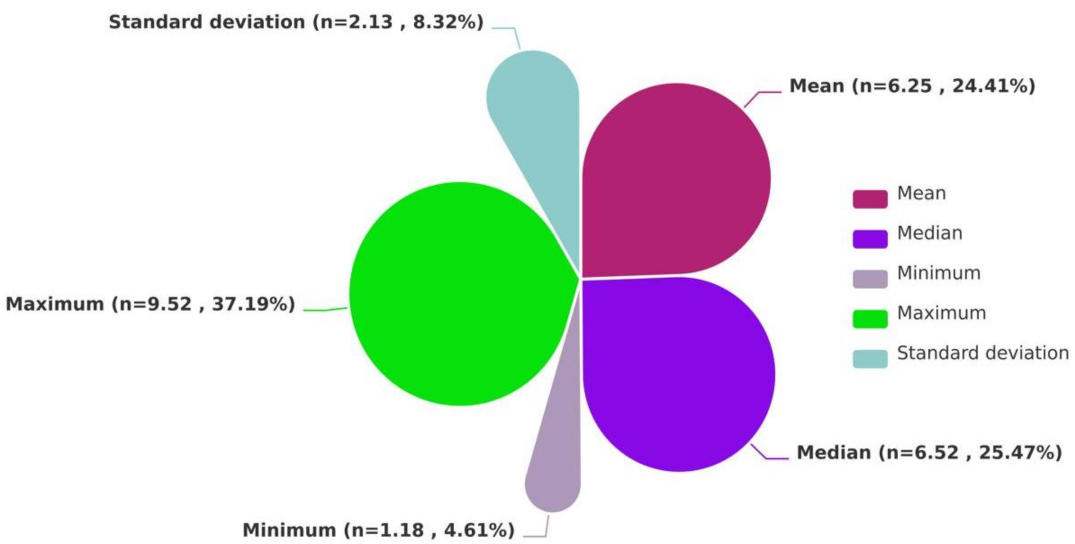

Figure 5: Impact of Water Uptake on Rainbow Trout Ova Diameter: The majority of ova diameter increases fall within a cluster around the mean value, indicating that most eggs experience a similar increase in diameter after water hardening.

Factors influencing egg mortality and management strategies

During the present study, it was revealed that both dead eggs and bloodworm (Chironomidae: Diptera) infestation led to Saprolegnia spp. Infection in the eggs. A single bloodworm could clump together and damage 4-6 healthy ova, ultimately causing significant mortality.

Discussion

Rainbow trout are typically spring spawners, but hatchery-reared stocks may breed in winter months as reported by (Okumuş, 2002). Off-season trout production can be achieved through photoperiod manipulation. The spawning period can even vary within the same region depending on water temperature and other geographical factors in Chitral, warmer areas like JTH experience earlier spawning (late October/early November) compared to colder locations like Golain Valley and Bamburate, where spawning occurs in late December.

Water temperature plays a crucial role in trout breeding and incubation. Higher temperatures shorten the incubation period. Eyed-ova appear at around 150 degree days (D°), with hatching occurring between 290-340 D° (Huet, 1986; Jiang *et al*., 2021). Other studies support this range. Jokumsen and Svendsen (2010) observed the eyed-ova stage at 180-200 D° (26-29 days after fertilization at 7°C) and hatching at 300-350 D°. Our study also confirms these findings. The eyed stage began at 155±5 D°, and the incubation period at 13.3°C to 12.7°C ranged from 305±5 D° to 349±5 D°, falling within the established range.

Efficient fish hatchery management is crucial for intensive fish culture, and the production of fish seed for stocking is essential for productive aquaculture (Haider *et al*., 2024). Rainbow trout eggs are highly sensitive and require meticulous handling during stripping. Water quality parameters significantly impact egg survival. Fluctuations in water temperature, low temperatures, and suspended solids can contribute to mortality rates reaching up to 50% (Okumuş, 2002). In the present study, the egg survival/hatching rate was 79% (3,395 eggs), while the mortality rate was 21% (916 eggs). Notably, 59% (544 eggs) of the total mortalities occurred before the eyed stage, and 41% (372 eggs) occurred afterward. The highest daily mortalities were observed on days 9, 8, and 10 after fertilization, with 222, 127, and 65 dead eggs collected, respectively. A significant positive correlation was found between egg mortality and temperature in rainbow trout breeding. Higher temperatures were associated with increased mortality rates. This is likely due to thermal stress negatively impacting embryonic development. Maintaining optimal water temperatures during incubation is crucial for maximizing egg viability and hatching success in rainbow trout breeding programs.

Rainbow trout produce a relatively small number of eggs compared to other culturable fish species. Generally, smaller females produce more numerous, smaller eggs (Quinn, 2005). Studies report varying ranges of relative fecundity, with Jokumsen and Svendsen (2010) estimating 1500–2000 eggs/kg. Okumus (2002) reported absolute fecundity ranging from 1509-9244 eggs for broodstock weighing 726-3231 grams, with a range of 1-4.2 eggs per gram. The current study on the broodstock at JTH revealed absolute fecundity ranging from 806-1980 eggs for fish weighing 300-856 grams, with a relative fecundity of 1.87-3.42 eggs/gram. This study observed that a 376g brooder produced 887 eggs, while a 400g brooder produced 806 eggs.

Research supports the observed relationship between body weight and fecundity. Springate (1985) found that total fecundity and egg size increase with fish size, while relative fecundity decreases (Yousuf and Razak, 2022; Njue *et al*., 2024). The present study confirmed a significant positive correlation between egg body weight and absolute fecundity, highlighting the importance of egg size in fecundity outcomes. Additionally, findings from the present research align with other studies indicating that egg production is influenced by various intrinsic and extrinsic factors, such as water temperature (Baki *et al*., 2021; Woynarovich *et al*., 2011), Genetic makeup (Springate, 1985), Age of spawners (García-Mondragón *et al*., 2021), quality and quantity of feed and management practices (Woynarovich *et al*., 2011).

Rainbow trout eggs undergo a process called water-hardening after fertilization, which involves the sealing of their pores, transforming them into what is known as Green Ova. These green eggs are delicate and susceptible to damage due to their newly hardened state. The hardening process is crucial for protecting the developing embryos within the eggs and preparing them for subsequent embryonic growth stages. Studies have shown that during water-hardening, rainbow trout ovum can increase in volume by up to 20% from their original size, with the process typically taking about 20 min (Poole *et al*., 2002). Water-hardened Ova are eggs that have completed the water-hardening process, crucial for shielding the developing embryos within the eggs before advancing to subsequent embryonic growth stages (Lahnsteiner and Patzner, 2002). The present research observed a significant increase (p-value = 0.0001) in the diameter of Green Ova compared to Water-hardened Ova, indicating a statistically significant difference and aligning with previous research on trout egg volume increase during water-hardening.

In several instances, sediments accumulated at the bottom of the external basket of the incubation tray can provide a habitat for hatching of viable insect eggs, such as chironomid bloodworms. These bloodworms, upon emerging from the sediments, can come into contact with fish ova, causing mechanical shock and making the ova susceptible to Saprolegnia spp. infection. Sunlight and fluorescent lights can adversely affect egg viability during stripping and incubation. Using a mobile torchlight for egg handling and covering horizontal flow incubators with windowpanes on the northern side can mitigate these negative effects. Careful monitoring and preventive measures are vital for successful fish farming practices. The study recommends the safe use of mobile torchlight for egg handling and installing window panes on the northern side of hatching rooms to minimize direct sunlight exposure during incubation.

Conclusions

The study conducted at Jaghour trout hatchery, Chitral Khyber Pakhtunkhwa, Pakistan, focused on the artificial breeding success, incubation period, and fecundity of Rainbow Trout *Oncorhynchus mykiss* (Kamloops Strain). The research emphasized the vital role of artificial breeding and spawning in trout culture for local communities with limited resources. It highlighted the necessity for comprehensive research in selective breeding, feeding, and gamete quality assessment for artificial fertilization, disease diagnosis, and broodstock management to obtain high-quality eggs and improved strains.

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Conflicts of interest

Authors declare no conflict of interest

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