



Age-Dependent Changes on Reproductive Potency of Female Rainbow Trout (*Oncorhynchus mykiss*, Walbaum 1792) in Kashmir

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Abstract

Rainbow trout, *Oncorhynchus mykiss* is extensively cultured in the Himalayas owing to its hardy nature, suitability for sport fishing, high meat quality and ease of breeding. In this study, the age-dependent reproductive performance of female rainbow trout specimens collected from Trout fish farms of Kashmir, were studied with the aim to gain insights into its breeding characteristics and to inform the development of an optimal broodstock management plan. The age of the specimens ranged from 0⁺ to 3⁺ years. Fish age showed a significant correlation with total length, total weight, ovary length, ovary weight and GSI. Fecundity was only observed in age 3⁺. The absolute fecundity of the fish in age 3⁺ ranged from 320 to 585 eggs, while relative fecundity ranged from 0.873 to 1.703 eggs/g body weight and the ova diameter recorded ranged from 3.11 to 4.68 mm. Histological examination showed that ovaries were in the early stage of development in age 0⁺; contained young oocytes in age 1⁺; exhibited oocytes at different developmental stages in age 2⁺; and were fully mature with oocytes at the yolk deposition stage in age 3⁺. Based on GSI values, ova-diameter and histological examinations, age 3⁺ was identified as the spawning age group.

Keywords: Age, fecundity, gonadosomatic index, histology, *Oncorhynchus mykiss*, ova-diameter

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Introduction

Salmonids are among the most important cultured fish species worldwide, (Lee & Donaldson, 2001), and among the salmonids, rainbow trout is particularly popular due to its appeal in sport fishing, excellent meat quality, and ease of propagation. It is a hardy species, capable of tolerating a wide range of environmental conditions, and readily accepts artificial feed, making it highly suitable for aquaculture. As a eurythermal species, trout can withstand considerable temperature fluctuations, has a shorter egg incubation period, exhibits rapid growth, and demonstrates greater resistance to most diseases (Jan et al., 2025). The unique environmental features, topography, and climatic conditions of Kashmir make the region especially well-suited for the breeding, rearing, and production of trout.

It is well recognized that the use of high-quality gametes from fish broodstock is crucial for ensuring the production of viable hatchlings in aquaculture practices (Kjorsvik, Mangor-Jensen, & Holmefjord, 1990; Billard, Cossin, Crim, & Suquet, 1995; Yaron, 1995). Studies have shown that the age of fish significantly influences gamete quality and, consequently, the survival rate of hatchlings. In rainbow trout, for instance, older and larger females tend to produce larger eggs than younger and smaller ones (Gall, 1974). Egg size, i.e., egg diameter increased with increase in age of rainbow trout (Bhandari, Regmi, & Sharad, 2025). Therefore, careful selection and management of broodstock are essential for enhancing the reproductive success and productivity of cultured fish (Rashid et al., 2024). In culture systems, trout do not spawn naturally, therefore, juveniles for stocking are obtained either through artificial spawning in incubation facilities or by collecting eggs from wild stocks. However, intense selection for production traits within closed and

relatively small broodstock populations of rainbow trout can result in considerable levels of inbreeding (D'Ambrosio et al., 2019). This, in turn, leads to inbreeding depression, negatively affecting female body weight, spawn weight (Kincaid, 1983), fecundity, and spawning age (Su, Liljedahl, & Gall, 1996). Consequently, evaluating the performance of reproductive characteristics in female trout is essential, as these traits represent a critical link between the spawning stock and subsequent recruitment (Gundersen, Nedreaas, Kjesbu, & Albert, 2000; Shamspour & Khara, 2016). Accurate assessment of size-at-age and age-at-maturity is essential for effective broodstock management, conservation of fishery resources, and optimization of aquaculture practices (Mendoza, 2006).

The present study investigated young rainbow trout with the objective of determining the age and size at first sexual maturity and to evaluate age-related variation in key reproductive parameters, including fecundity, gonadosomatic index (GSI), and ova diameter. Ovarian developmental stages were also characterized through histological analysis. Accordingly, this study aimed to establish baseline data on early reproductive development to provide site-specific insights that support sustainable broodstock management and aquaculture advancement in the Himalayan region.

Materials and Methods

A total of 120 female specimens, 30 from each age group obtained from Mammam and Laribal trout fish farms of Kashmir were used for the study.

Fish age was determined from opercular bones, a well-established and reliable method in temperate waters (Le Cren, 1947; Mann, 1973). Briefly, opercular bones were carefully removed from freshly collected specimens, boiled briefly in water to detach adhering tissues, and treated with 50% H₂O₂ for approximately 15 minutes. They were then rinsed with water and sun-dried for three days. Annual growth rings on the dried opercular bones were examined and counted to estimate fish age (Qasim, 1973).

Fecundity was estimated from preserved ovaries using the sub sampling method.

Absolute fecundity (A.F) was calculated using the following equation:

$$A.F = \frac{\text{No. of ova in sub-sample} \times \text{Total ovary weight}}{\text{Weight of sub-sample (g)}}$$

Relative fecundity (R.F), i.e., number of ova per gram of body weight was calculated by dividing absolute fecundity by total weight of the fish.

For gonadosomatic index (GSI) estimation, the body weight and ovary weight of each specimen were recorded. GSI was calculated as the percentage of gonad weight relative to total body weight, and used to assess maturity and determine the breeding cycle of the fish (Afonso-Dias, Reis, & Andrade, 2005).

$$\text{Gonadosomatic index} = \frac{\text{Gonad Weight}}{\text{Body Weight}} \times 100$$

The diameter of intra-ovarian eggs collected from preserved ovaries, was measured using a digital Vernier caliper following Clark (1931). A total of thirty ova were examined and measured from ovaries of 30 fishes aged 3⁺, resulting in 900 ova measured during the study.

Histological examinations were performed to study ovary development. Ovaries preserved in 10% formalin were sectioned into 1–2 mm pieces and washed overnight under a gentle flow of tap water. The tissues were dehydrated through a graded ethanol series, cleared in xylene (A.R.), and embedded in paraffin using standard impregnation techniques. Sections of 5 μm thickness were prepared with a microtome and stained with 2% hematoxylin and eosin. Photomicrographs were captured using a Magnus DC10 camera mounted on a Magnus MLX microscope and examined to identify different stages of ovarian development

The data were tabulated and analyzed using both descriptive and inferential statistical methods with the aid of standard software packages, including PAST and Microsoft Excel. A one-way analysis of variance (ANOVA) was used to test for statistically significant differences in reproductive parameters among age groups. Correlation was estimated between age and total length, total weight, ovary length, ovary weight and GSI of rainbow trout.

Results and Discussion

Age and growth studies are important for addressing common issues in fishery management (Polat, Bostanci, & Yilmaz, 2001). Knowledge of age

structure of fish populations allows estimating growth rates, mortality, and recruitment, which are essential for calculating population production rates (Hilborn & Walters, 1992; Chung & Woo, 1999). Fish have several calcified structures like otoliths, scales, vertebrae, spines and opercular bones that can be used for age determination and growth parameter estimation to assess the age composition of exploited fish populations and stocks (Panfili, de Pontual, Troadec, & Wright, 2002). As the scales of rainbow trout are very small, the age was estimated by counting annual growth rings on operculum and it ranged from 0⁺ to 3⁺ years, with total length ranging from 115–158 mm to 246–392 mm and weight ranging from of 18–40 g to 265–630 g. Previous reports have noted a maximum age of up to 11 years for rainbow trout (Froese & Pauly, 2009). Strong seasonal temperature fluctuations in temperate waters result in the annual formation of alternating opaque and translucent growth zones, producing clearly defined annuli that are more distinct in temperate fishes than in tropical species (Nikolskii, 1963; Bhat, Balkhi, Najar, Shah, & Khan, 2013).

A strong positive correlation was recorded between age and total length ($r = 0.893$), age and total weight

Table 1. Correlation (r value) between age and total length, total weight, ovary length, ovary weight and GSI of *O. mykiss*.

Traits	Age (N=120)	p- value
Total Length (mm)	0.893	< 0.01
Total Weight (g)	0.948	< 0.01
Ovary length (mm)	0.878	< 0.01
Ovary weight (g)	0.749	< 0.01
GSI (%)	0.753	< 0.01

Table 2. Comparative analysis of mean total length, total weight, ovary weight and GSI in 4 age groups of *O. mykiss*.

TRAITS	Mean±SE				p-value
	Age 0 ⁺ N=30	Age 1 ⁺ N=30	Age 2 ⁺ N=30	Age 3 ⁺ N=30	
Total length (mm)	130.0±1.75	244.1±2.31	275.3±3.95	302.9±5.73	< 0.01
Total weight (g)	28.1±1.00	172.3±4.03	249.0±7.31	424.0±13.53	< 0.01
Ovary weight (g)	0.06±0.00	0.27±0.00	0.59±0.01	44.23±2.07	< 0.01
Ovary length (mm)	36.2±0.69	48.1±0.83	59.6±1.16	132.0±2.24	< 0.01
Gonadosomatic index (%)	0.23±0.01	0.15±0.00	0.24±0.00	10.44±0.39	< 0.01

($r = 0.948$), age and ovary length ($r = 0.878$), age and ovary weight ($r = 0.749$), and age and GSI ($r = 0.753$) of female rainbow trout (*O. mykiss*) (Table 1). Statistical analysis using one-way ANOVA revealed a significant difference in all the parameters among different age groups of trout ($p < 0.01$) (Table 2). All measured parameters, including total length, total weight, ovary length, and ovary weight, increased consistently with advancing age (Fig. 1–4). Notably, rainbow trout attained marketable size (250 g) by age 2⁺.

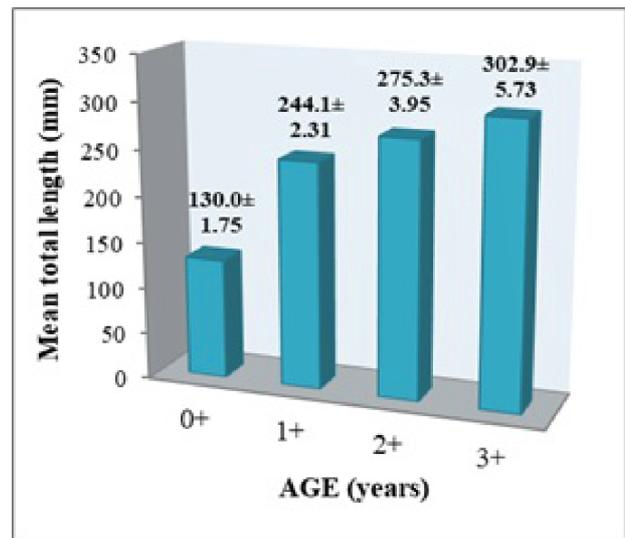


Fig. 1. Relationship between age and mean total length of *O. mykiss*

Fecundity in *O. mykiss* was studied by examining 30 females aged 3⁺. The highest absolute fecundity recorded was 585 eggs for a fish measuring 319 mm in total length and weighing 420 g, while the lowest value was 320 eggs for a fish measuring 246 mm and weighing 355 g. The mean ± SE value of absolute fecundity and relative fecundity were 477.73 ± 13.06 eggs and 1.142 ± 0.032 eggs/g respectively. Relative

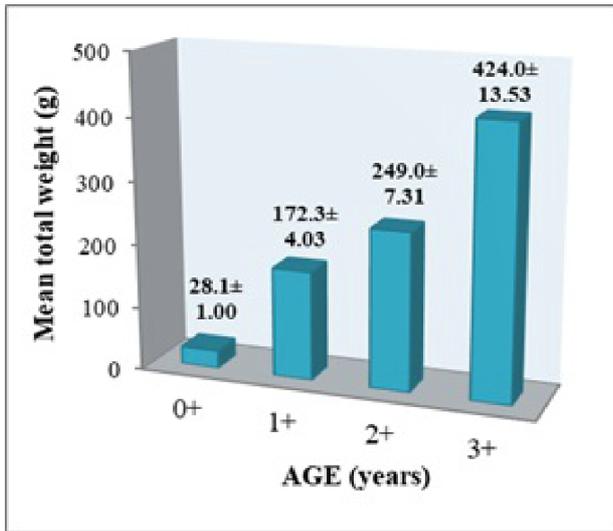


Fig. 2. Relationship between age and mean total weight of *O. mykiss*

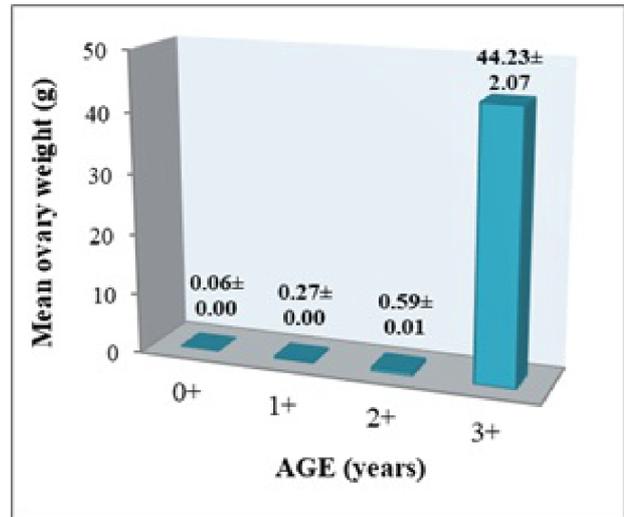


Fig. 4. Relationship between age and mean ovary weight of *O. mykiss*

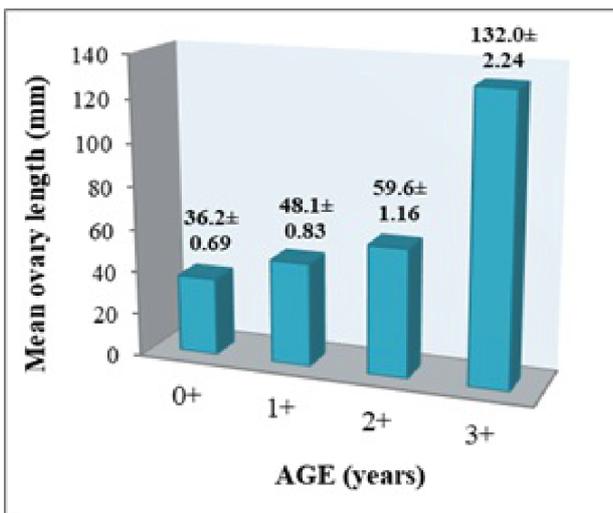


Fig. 3. Relationship between age and mean ovary length of *O. mykiss*

fecundity ranged from 0.873 to 1.703 eggs/g of body weight. The relationship between fecundity (F) and ovary weight (OW) and ovary length (OL), of age group 3+ along with their R² values has been established are as follows:

The relationship between fecundity (F) and ovary weight (OW) and ovary length (OL) were established as follows:

$\text{Log}_{10} F = 1.9476 + 0.4453 \text{ Log OW}$, R²=0.5529 (Fig. 5)

$\text{Log}_{10} F = 0.671 + 0.9454 \text{ Log OL}$, R²=0.3484 (Fig. 6)

Within a species, it has been found that fecundity can vary based on latitude and location (Cushing, 1968; Mann, Mills, & Crisp, 1984), and also with spawning periods (Ware, 1975). Scott and Crossman (1973) reported that fecundity in salmonids is highly variable and depends on brood size. In broodfish management, fecundity is an especially important trait as farmers prefer to maintain fecund broodfishes to maximise egg production (Serezli, Guzel, & Kocabas, 2010). Fecundity is affected by age, size,

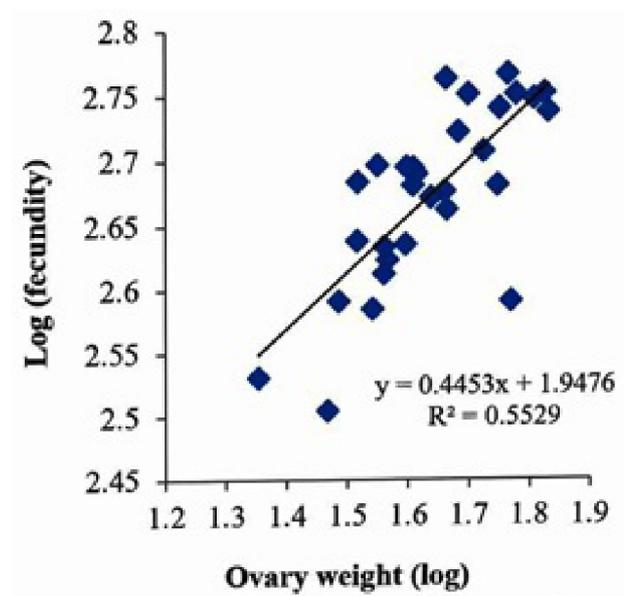


Fig. 5. Logarithmic relationship between fecundity and ovary weight of *O. mykiss*

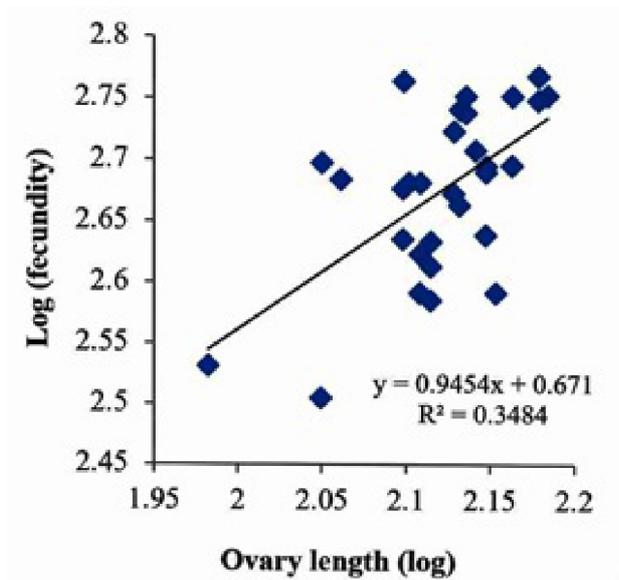


Fig. 6. Logarithmic relationship between fecundity and ovary length of *O. mykiss*

fish species, feeding, season and environmental conditions (Nikolskii, 1969; Thorpe, Miles, & Keay, 1984). In the present study, eggs (ova) were observed only in the 3⁺ age group. The mean absolute fecundity was 477 eggs, and mean relative fecundity was 1.14 eggs/g in age 3⁺ *O. mykiss*. The females attained maturity after their third year, consistent with findings from other studies. Joshi (2009) studied brood stock maturity and artificial breeding of *O. mykiss* and recorded fecundity ranging from 547–1402 eggs per kg body weight. Rasool and Jan (2013) also reported a positive correlation of fecundity with total fish length, fish weight, ovary weight and ovary length in *Salmo trutta* fario. In fishes, it is observed that body weight is more related with the egg production capacity than that of body length (Wootton, 1973).

It was noticed that the mean GSI values initially decreased and then increased from age group 0⁺ to 3⁺. The minimum mean GSI value of 0.15% was observed in age group 1⁺ and maximum mean GSI value of 10.44 was observed in age group 3⁺. The mean±SE values of GSI in different age groups of trout are shown in Fig. 7. A scattergram plotted between GSI and ovary weight of age group 3⁺ showed a significant positive correlation between the two parameters (Fig. 8), indicating that GSI increases with ovary weight.

The gonadosomatic index (GSI) serves as an important indicator of fish spawning activity in fish across both temperate and tropical regions. It reflects gonadal development and maturity, typically increasing as the fish matures and declines abruptly after that (Parameswaran, Alikunhi, & Sukumaran, 1972). In this present study on female *O. mykiss*, GSI ranged from a minimum of 0.09% in a fish of age 0⁺ to a maximum of 15.72% in a fish of age 3⁺. The rapid increase in GSI is due to the accumulation of yolk, which serves as the nutritional reserve for the developing embryo (Wiegand, 1996). Based on GSI values and gonadal conditions, age 3⁺ was identified as the primary spawning age group among the four age groups studied. The lowest GSI values were found in younger age groups as during the early years, the gonads were in developing phase and were not fully mature. The highest GSI values in age 3⁺ may be attributed to the growth rate of ovary which is in confirmation to other studies on rainbow trout and indicates that considerable amount of energy is required to complete the reproductive cycle (Scott & Sumpter, 1983). Tyler, Sumpter, and Witihames (1990) reported that the indices of ovarian growth (gonad weight and oocyte size) increased dramatically during vitellogenesis and GSI underwent almost a 50-fold increase, from 0.4% to 20%, reaching a peak just before ovulation in *O. mykiss*. In the present study, the GSI also recorded almost a similar increase of 50-fold from 0.23% to 10.44%.

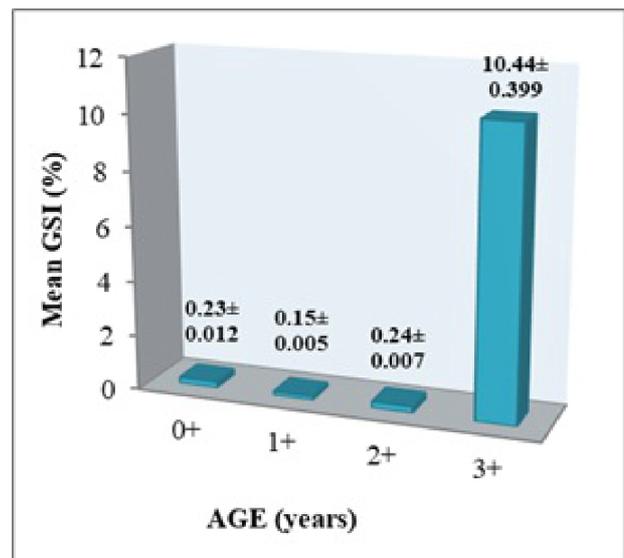


Fig. 7. Relationship between age and mean GSI of *O. mykiss*

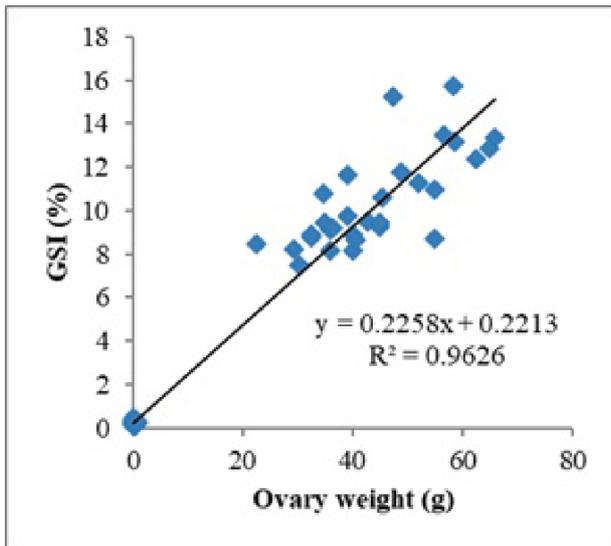


Fig. 8. Relationship between GSI and ovary weight of *O. mykiss*

For the study of ova diameter, 900 eggs were examined from 30 female specimens of *O. mykiss* aged 3⁺ (30 eggs from each specimen). The ova showed a wide range of size variation and were divided into four batches of 3–3.5 mm, 3.5–4 mm, 4–4.5 mm and 4.5–5 mm diameter. The overall egg diameter ranged from 3.11 mm to 4.68 mm, with a mean value of 3.75 mm. Majority of the oocytes (61.7%) were observed in the 3.5–4.0 mm size range, 19.7% oocytes in the 3.0–3.5 mm range, 14.2% oocytes in the 4.0–4.5 mm range and 4.2% oocytes in the 4.5–5.0 mm range (Fig. 9).

Ova diameter studies are an integral part of fish biology and is a vital tool in the classification of maturity stages in teleosts. Clark (1931) and Hickling and Rutenberg (1936) reported that ova-diameter studies can help determine the time and frequency of spawning in fishes. The demersal eggs of rainbow trout are relatively larger compared to those of indigenous snow trout inhabiting the Kashmiri waters, which contributes to their lower fecundity. In the present study, the ova-diameter of age 3⁺ varied from 3.11 mm to 4.68 mm with a mean value of 3.75 (± 0.01) mm. A unimodal distribution of ova was observed in the fish. There was synchronous development of the oocytes suggesting the fish to be an iteroparous total spawner. Tyler et al. (1996) documented that *O. mykiss* produce a single batch of eggs annually, with all ova developing and being ovulated simultaneously, the oocyte

size increases from less than 0.5mm to 4–5 mm in diameter.

Bara (1960) reported that in *O. masou* all the oocytes develop together at the same time and fishes spawn only once in a life time. Sharma and Bhat (2014) have also reported that most ovarian follicles in rainbow trout are at synchronous stage of development and are single season spawners. The pattern of ova distribution, whether unimodal or bi-modal is an important tool to understand spawning cyclicality in fishes. This distribution reflects the pattern of oocyte growth, which occurs predominantly during the vitellogenic phase of development. During vitellogenesis, oocytes undergo rapid growth, with their diameter increasing from approximately 1 mm to 5 mm. This phase therefore accounts for more than 98% of the final oocyte volume (Tyler et al., 1990). Oocyte growth during vitellogenesis is achieved mainly through the uptake of extra-ovarian proteins from the bloodstream, which are subsequently deposited as yolk within the developing oocytes (Tyler, Sumpter, & Bromage, 1988). Tyler et al. (1996) also documented that *O. mykiss* produces a single batch of eggs each year and has synchronous growth of oocytes where all ova are ovulated at the same time. Normally there are two phases of oocyte growth in trout, primary growth and secondary growth, which is further divided into number of stages (Nagahama, 1983; Tyler, 1991; Tyler, Nagler, Pottinger, & Turner, 1994). However, Tyler et al. (1996) stated that growth principally occurs in secondary growth phase during vitellogenesis when oocyte size increases from less than 0.5 mm to 4–5 mm in diameter.

Histological examinations during the present study revealed that the ovaries of *O. mykiss* were in different developmental stages (Fig. 10).

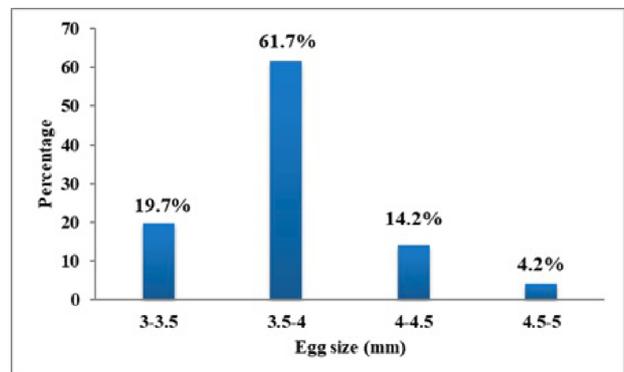


Fig. 9. Egg size distribution of Age 3⁺ of *O. mykiss*

Ovary structure of Age 0⁺: Ovaries were in an immature stage, small, slender, thread-like, and pale in colour. Histological examination showed ovigerous lamellae containing nests of oogonia (NOO). Non-yolky primary oocytes develop from these oogonial cells after a series of mitotic divisions. Ovary weight in this group ranged from 0.04–0.09 g.

Ovary structure of Age 1⁺: There was a slight increase in size and weight of ovaries. Ova were invisible to naked eye in this age group. Histological examination showed that each oogonium increased little in growth and young oocytes (O) in large numbers were visible under the microscope. Ovary weight ranged from 0.2–0.38 g in this group.

Ovary structure of Age 2⁺: Ovaries further increased in size and weight, becoming increasingly vascularised. Histological examinations revealed ova were small and characterized by the appearance of yolk vesicles in the form of minute granules in the extra vesicular ooplasm. During this stage, the ovary was filled with previtellogenic oocytes in various developing stages of yolk deposition. Ovary weight ranged from 0.49–0.85 g.

Ovary structure of Age 3⁺: In this age group, the ovaries reached their maximum weight occupying almost the entire body cavity and were filled with highly developed vitellogenic oocytes. Histological examinations showed oocytes were filled with yolk granules (YGr) and yolk globules (YG). Ova were turgid, deep yellow in colour, large sized and visible to the naked eye through the thin and transparent ovarian wall. The zona pellucida (ZP), the egg membrane, was clearly visible. The fish appeared gravid, with a rounded abdomen due to the accumulation of mature ova.

Histology is a powerful tool in reproductive studies and is extensively used for sex verification and assessment of reproductive phase (Blazer, 2002). Histologically, the wall of ovary is made up of three layers; the outermost thin peritoneum, a tunica albuginea comprised of connective tissue, muscle fibers and blood vessels, and the innermost layer called germinal epithelium which projects into ovarian lumen in the form of lamellae (Wali, Shah, Bhat, & Mohd, 2022). During the present study, various developmental stages of ovaries were clearly observed. The ovaries of the immature female trout were initially seen as granular rings lying in the body cavity dorsal to the gut. As they develop and

eggs begin to form, the ovaries transform into bilateral masses, positioned above the intestinal tract. Each ovary is contained within a thin membrane which ruptures when the female is ripe so that the eggs become loose in the body cavity and can be squeezed out through the vent (Vass, 2000).

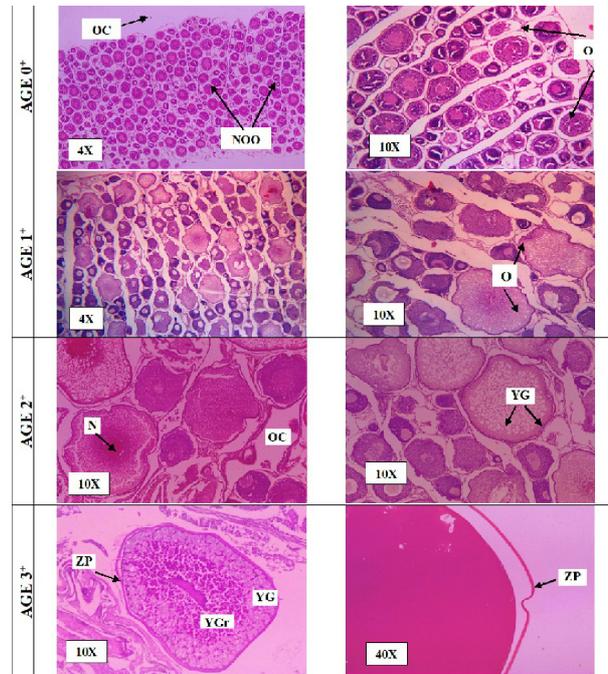


Fig. 10. Histological structure of ovaries of 0⁺, 1⁺, 2⁺ and 3⁺ age groups of *O. mykiss*

Based on the findings of the present study, it can be concluded that the rainbow trout population examined was in good physiological condition, suggesting that appropriate scientific management and husbandry practices are being followed. The fish attained a marketable size of approximately 250 g by the 2+ age group. Under both experimental and commercial conditions, females belonging to the 3+ age group are routinely stripped for egg collection. However, the results of this study indicate that this practice requires refinement, as egg quality is likely to depend on the physiological condition and reproductive maturity of individual fish. Accordingly, only healthy and fully mature females should be selected for stripping. Based on gonadosomatic index (GSI), ova diameter measurements, and histological observations, the 3+ age group was identified as the mature spawning cohort of female rainbow trout, making it the most suitable age group for broodstock selection and breeding programmes in the Kashmir region.

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