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Comparative growth performance of diploids and triploids rainbow trout (*Oncorhynchus mykiss*)

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Abstract
Due to extra genetic material, triploid fish remain more heterozygous and suppose to be useful for increasing growth. A field study of 6 months was conducted to evaluate the growth performance of the diploid and triploid *Oncorhynchus mykiss,* which is one of the candidate fish for coldwater aquaculture in Himalayan region. Triploids were produced by pressure shock after post fertilization of eggs. The control group of diploids was observed for final body length and weight from the range of 32.9±0.83mm to 33.0±0.84 mm (0.89±0.083g to 0.91±0.081g), while treated triploids were observed as 42.8±0.79 to 43.25±0.77 mm (1.05±0.033 to 1.07±0.033 g). Observed data showed a significant difference (p<0.05) with 17.58% higher growth in triploids over diploids. Survival rate was in the range of 94-96% without any significant difference in both the groups. Study showed feasibility for achieving better growth of this fish in captive rearing with triploids.

Keywords: *Oncorhynchus mykiss*, triploidy, heterozygous, post fertilization

Introduction
The rainbow trout is a prominent coldwater fish classified as *Oncorhynchus mykiss* and belongs to the family Salmonidae. It is recognized as the most widely farmed trout in the world and is popular as a sport fish and an experimental fish. It thrives in highly oxygenated freshwater with temperatures of 13-18 °C. These characteristics establish them as one of the most widely introduced and cultured fish across the globe and its farming is also prominent in the coldwater regions of India. Nevertheless, genetic fatigue, slow growth, early maturity and low productivity are limiting the expansion of trout production. A viable strategy that could overcome these farming constraints in large scale operation is the production of triploid fish that are sterile and more heterozygous. Such chromosomal manipulation and generation of polyploids is not exclusive to fish. Many plants used in modern agriculture are induced polyploids, selected to increase productivity associated with greater cell size or disease resistance. Triploid sterile fish are beneficial in aquaculture due to extra genetic material and more heterozygocity. In triploids, most of the anabolic energy is transferred to somatic growth [1]. Somatic growth is one of the most fundamental biological processes required for survival and thus has important fitness consequences, and growth rate is frequently used as an indicator of the capacity to acquire food resources [2, 3]. Triploidy is characterized by the change in normal diploid (2n) set of chromosomes to the state of triploid (3n) with an additional set of chromosome [4, 5, 6, 7, 8]. As compared to diploids, triploid cells are relatively big and will have a larger nucleus, however the ratio between the cytoplasm and the nucleus is constant [9, 10]. Increased cell size in triploids applies to all tissues and cells of the body. Several production related differences have been observed between triploid and diploid with respect to survival and hatchery performance [11], growth and harvest quality [12, 13], feeding behaviour [14], nutrient requirements [15, 16], body composition and energy reserves [17]. Based on the above reviews, the purpose of the study was to investigate the effect of triploidization on growth performance of triploid with their diploid counterparts in rainbow trout in field condition.

Materials and Methods
The study was carried out at ICAR-Directorate of Coldwater Fisheries Research (DCFR), situated at Bhimtal (Latitude 290 21’N, Longitude 790 34’E, 1370 masl), Uttarakhand.
For the experiment purpose triploids were produced by pressure shock at 9500 psi for 5 min. after 24min. of post fertilization [18] and reared up to fingerling size.

Healthy fingerlings having the size of 12-13 g (n=60) of both, triploid and diploid stocks were reared for 6 months in duplicates under flow through system in FRP tanks with dimensions of 1.5×1.0×1.0m. It was a continuous water flow system where the flow (2-3 LPS) was regulated using inlet and outlet. The water in the system was showered from the inlet and outflow pipe was placed by drilling at a height of 0.67m from the bottom so that a constant level of water ($1m^3$) was maintained in the tanks. Fish were fed with experimental diet containing 50% protein and 15% lipid, twice a day @4-5% of their body weight. Important water quality parameters such as dissolved oxygen concentration/saturation, temperature, pH, alkalinity total ammonia nitrogen and nitrite concentrations were regularly monitored and recorded [19]. Initial length and weight of fingerlings was recorded for both the groups in duplicates. Growth indices and survival was calculated by following formulae-

Body weight gain (g) = Mean final weight (g) – initial weight (g)

$$\text{Percentage weight gain} = \frac{\text{final weight(g)} - \text{initial weight(g)}}{\text{initial weight(g)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fishes harvested}}{\text{Initial number of fishes}} \times 100$$

$$\text{SGR (\%day}^{-1}\text{)} \frac{= \text{loge Final weight} - \text{loge initial weight}}{\text{number of days}} \times 100$$

Net yield (g/m^3) = total fish weight harvested (g) - Total initial weight (g)

$$\text{Feed conversion ratio} = \frac{\text{feed given (dry weight in grams)}}{\text{body weight gain(wet weight in grams)}}$$

$$\text{Feed efficiency ratio} = \frac{\text{body weight gain (wet weight in grams)}}{\text{feed given (dry weight in grams)}}$$

$$\text{Percent return to variable cost} = \frac{\text{Net profit}}{\text{Total variable cost}} \times 100$$

$$\text{Benefit cost ratio} = \frac{\text{Total return}}{\text{Total cost}}$$

Descriptive statistics of the data were calculated by using computer software Statistical Package for Social Science (SPSS Version 22.0).

Results

The growth performance of tested fish groups are shown in table-1 & 2 and Fig. 1 & 2, which showed that the triploid growth was significantly higher ($p<0.05$) than the diploid. The observed final body length and weight data reflect the 20.2% better growth and 19.0% better yield of triploids over diploids. The net weight gain in 6 months was calculated as 145.04±6.54g for diploids and 192.23±6.21g for triploids. In triploids, better SGR, lower FCR, higher FER reflects superior growth traits in field condition. Almost similar survival (96-97%) was observed in both the groups without significant difference. Comparatively, 107.2 percent return on variable cost and higher benefit cost ratio in indicates the economic feasibility of triploids culture. The water temperature in all the replicates was almost similar ranging from 14-20 °C. Dissolved oxygen was ranged from 7.8-8.6 mg/l, pH 7.2-7.4, free CO_2 0.5-2.5mg/l, total alkalinity 104-112 mg/l. Ammonia–N and nitrite-N level was recorded as >0.01 in all the replicates.

Table 1: Average net weight (g) and length gain (cm) ± SD of diploid and triploid rainbow trout in 6 months rearing

Treatment	Initial avg. length (cm)	Final avg. length (cm)	Initial avg. weight (g)	Final avg. weight (g)	Avg. Net length gain (cm)	Avg. Net weight gain (g)
Diploids	10.60±0.46	27.76±0.58	12.88±0.94	172.80±6.56	17.16±0.88	159.92±6.54
Triploids	10.80±0.24	30.20±0.56	13.40±0.88	205.63±6.56	19.40±0.58	192.23±6.21

Values are given in mean ± SD (n=2)

Table 2: Average growth, survival and production of diploid and triploid rainbow trout in 6 months rearing

Treatment	Avg. net length gain	Avg. net weight gain	SGR	FCR	FER	Average Survival rate	Net yield (kg)/ m^3
Diploids	17.16^a±0.72	159.92^a±6.56	1.90^a±0.18	1.58^a±0.00	0.64^a±0.00	96.66^a±0.00	9.28^a±0.32
Triploids	19.40^b±0.54	192.23^b±6.26	2.07^b±0.14	1.46^b±0.03	0.71^b±0.01	95.83^a±0.00	11.05^b±0.39

Values are given in mean ± SD (n=2)

Table 3: Economic analysis of diploid and triploid rainbow trout in 6 months rearing

Variable cost					
expenditure	**Rate (₹)**	**Quantity/m^3**		**Cost indian rupee(₹)**	
		diploids	**triploids**	**diploids**	**triploids**
Fish seed (no.)	10/piece	60	60	600	600
Fish feed(kg)	100/kg	13.3	16.2	1330	1620
Misc. Expenditure				980	980
Total variable cost				2910	3200
Return					
production		Quantity kg/m3		Cost Indian rupee	
		diploids	triploids	diploids	triploids
Total fish production	600/kg	8.41	11.05	5046	6630
Net profit				2136	3430
Percent return to variable cost				73.4	107.2
BCR				1.73	2.07

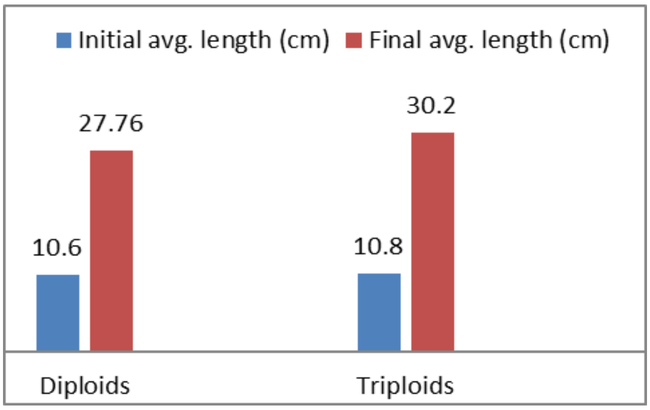

Fig 1: Initial and final Length of rainbow trout in 6 months rearing

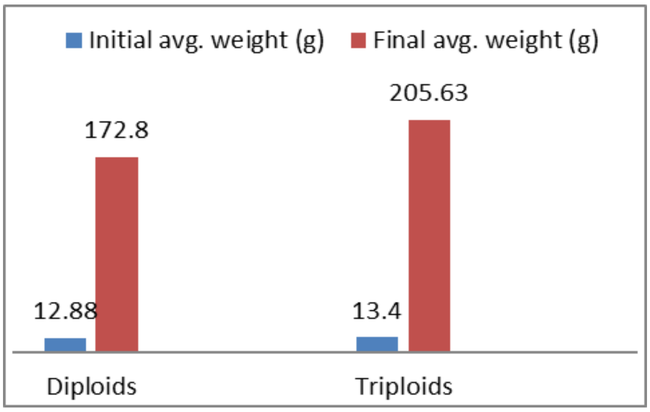

Fig 2: Initial and final weight of rainbow trout in 6 months rearing

Discussion

Growth rate is an important characteristic for the genetic improvement in fish for commercial production [20]. In general, triploids are functionally sterile due to the irregular meiotic division resulting in aneuploid gametes [21]. Hence, it is expected that triploids would retain a normal growth [22, 23]. Pradeep *et al*. (2012) [24]; Akhmad *et al*. (2020) [1] studied that the increase in triploid growth is due to the influence of sterility. Wang *et al*. (2015) [25] observed better growth in triploid salmon over the diploids. Triploids have a better growth rate than diploids at maturation in catfish and tilapia [26, 27, 28]. Better growth has been reported in triploids of Tilapia and European catfish at the age of 14 weeks [29, 30]. Higher growth rates of female triploid fishes have been shown for rainbow trout, *Oncorhynchus mykiss* [31, 32]. In contrast to these results the growth of juvenile triploid fish was found either to be the same as [33, 34, 35], or slower than that of diploids [36].

In Indian conditions, Joshi *et al*. (2005) [37] reported average growth as 200 g in 12 months at the thermal regime of 4.5-20 $^{\circ}$C. In another field study in central Himalaya, average growth was recorded as 300 g (range 260 - 400 g) at thermal regime of 5.0 – 22 $^{\circ}$C [38]. The reported generated information suggested that higher temperature in mid altitudes of Himalayas can help better yield of rainbow trout, provided farm management practices are optimized [39]. In Nepal, marketable size of 200 - 300 g reaches at 14 - 16 months of culture period with the stocking density of 50 fish/m^2 [40, 41]. Whereas the fish takes approximately 8 months to reach a market size of 300-350 g in trout farms of Idaho, U.S.A after being stocked in raceways when 4 inch long fingerling stocked [42]. With regard to stocking density, a flow rate of 4 LPS can support up to 20 kg/m^3 fish biomass. A minimum rate of 500 m^3 per day of water flow is necessary for 1 tonne of trout produced [43].

Conclusion

The study reveals that triploids of rainbow trout have superior growth traits which are beneficial for better aquaculture practice of this species in hills.

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