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Effect of heat shock on early survival rates of eggs in rainbow trout (*Oncorhynchus mykiss*) of Kashmir Himalayas

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Abstract
The aim of this study was to compare the effects on early survival rates of rainbow trout eggs exposed to heat shock shortly after fertilization. Four different treatments and a control group were used; each treatment group was replicated thrice. Heat shock at 26 ºC and 28 ºC for 10 min. duration were applied 15 min. and 20 min. after fertilization using a water bath. No treatment (heat shock) was given to Control. The highest fertilization rate among the treatment groups was observed in group T1 (26 ºC, 15 min. after fertilization) at 90.393±0.37%, while the lowest fertilization rate of 88.483 ± 1.68% was observed in group T4, heat shocked at 28 ºC, 20 min. after fertilization. However, the fertilization rate for the control group observed was 93.16±0.41%. It was found that the fertilization rate was higher in control group as compared to treatment groups. There was a significant difference (P<0.05) between the treatment groups and control group. The highest hatching rate among the treatment groups was observed in group T2 heat shocked at 26 ºC after 20 min. of fertilization (81.843±0.88%), while the lowest hatching rate o(72.153± 1.17%) was observed in group T3 heat shocked at 28 ºC after 15 min. of fertilization. The hatching rate was higher (84.28±0.46%) in control group as compared to treatment groups. It was observed that increasing heat shock intensity reduced the survival percentage of eggs from fertilization to hatching, however by increasing time after fertilization hatching rates also increased. Fertilization and hatching rates showed significant differences (P<0.05) between treatment groups and control group.

Keywords: rainbow trout, thermal shock, eggs, fertilization, hatching

Introduction
Rainbow trout, *Oncorhynchus mykiss*, is a cultivable fish that contributes significantly to upland aquaculture production in the region. It is commonly desired in upland aquaculture systems due to its superior growth in lower water thermal regimes, hardy nature, easy breeding procedure, optimal artificial feed intake, wide temperature tolerance, and high market price. (Vass, 2002) [23]. It is one of the most promising cold-water cultivable fish species and has a considerable scope for its expansion. It is a high value cold-water fish belonging to the Salmonidae family that thrives at temperatures between 10 ºC and 15 ºC. (Shah *et al*., 2009) [18].

Triploidy induction is a widely used technique for raising sterile fish for aquaculture and fisheries management (Thorgaard, 1986) [22]. Triploidy is triggered in fishes by blocking second polar body extrusion during the second meiotic division shortly after fertilisation of freshly stripped eggs, using various physical shocks and chemical treatments. In order to induce triploidy in many fish species such as: rainbow trout (Solar *et al*., 1984) [19], *Ctenopharyngodon idella* (Cassani and Caton 1985) [5]; *Cyprinus carpio* (Linhart *et al.*, 1991) [10]; red tilapia (Pradeep *et al.*, 2012) [13]; brown trout (Preston 2014) [14]; *Labeo rohita* (Aruljothi 2015) [1], a variety of techniques such as thermal shock, hydrostatic pressure shock, and chemical shock have been used.

Because of the ease of operation and the simplicity of the equipment used, thermal shock treatment is usually preferred. Heat shock is an efficient and commonly used technique for polyploid induction in fish, but it is not always 100 percent effective and may have negative consequences (Bazaz *et al*., 2020) [4]. Triploidy induction can be used as a starting point for further research into other shock protocols and ramping up the most practical approach for commercial triploid stock production. To ensure a full yield of progeny, the shock must occur during second meiotic division and must be sufficiently intense to disrupt the spindle fibres. As a result, shock frequency, duration, and application time must all be optimally combined.

Thermal shock such as cold shock and heat shock treatments (Sun *et al*., 1992; Yang *et al*., 1997; Pandian and Koteeswaran, 1998) [20, 24, 12] are considered safe for triploid induction as no harmful chemicals are used. The present study was conducted to compare the effect of four heat shock treatments and a control on the development of rainbow trout eggs in terms of fertilization and hatching rates.

Materials and Methods

Brood-stock collection and segregation

Healthy parent stocks of male and female rainbow trout were collected from Trout Culture Farm, Laribal, Srinagar (J&K Govt.). The parental brood stocks were fasted for 48 hours prior to sperm and egg collection during the pre-spawning season. The length of male rainbow trout ranged from 30.3cm to 45.1cm with a mean value of 38.77±1.38cm while as for female rainbow trout, the length ranged from 34.5cm to 47.4cm with a mean value of 38.05±1.32cm. The observed weight of male rainbow trout ranged from 623g to 1065g with a mean value of 794.6±49.3g while as the female rainbow trout weighed in the range of 635g to 1237g with a mean value of 766.3±64.3g.

Stripping and fertilization

As outlined by Bozkurt (2006) [2], the fertilization process was carried out using the dry stripping method. Manual stripping and gentle pressure on the abdomen were used to collect eggs and milt in clean, sterile, and dry plastic bowls. For fertilization, the eggs and milt were mixed with the help of a smooth, clean bird feather.

Heat shock treatment for triploidy induction

The eggs were divided into 4 treatment groups (replicated thrice) and a control group. Each replicate treatment group consist about 733 to 1536 eggs. Heat shock was given at 26 ºC after 15 minutes of fertilization for 10 minutes, to the first experimental unit (T1), while for second experimental unit (T2), heat shock was given at 26 ºC after 20 minutes of fertilization for 10 minutes. The third experimental unit (T3) was given heat shock at 28 ºC after 15 minutes of fertilization for 10 minutes while the fourth experimental unit (T4) was given heat shock at 28 ºC after 20 minutes of fertilization for 10 minutes to induce triploidy (Table1). For each temperature, the eggs were kept in a hot water bath maintained at the required temperature.

Table 1: Heat shock treatment details in *O. mykiss*

Groups	Recurrence number	Treatment	Shock Duration (Min.)	Time After Fertilization (TAF) (Min.)	Shock Temperature
T1	I II III	Heat	10	15	26 ºC
T2	I II III	Heat	10	20	26 ºC
T3	I II III	Heat	10	15	28 ºC
T4	I II III	Heat	10	20	28 ºC
TC (Control)	I II III	—	—	—	—

Incubation of Eggs

Following heat shock treatment, eggs were carefully placed in perforated hatching trays maintained with a constant supply of freshwater for incubation. The temperature of the water was checked regularly during the incubation period. Dead eggs were removed and counted on daily basis. Water temperature was recorded regularly and ranged between 8.3 ºC to 11.2 ºC. The fertilization rate was determined at eyed ova stage for each replicate of treatment group and control group. The eyed ova stage was reached after 16 to 21 days of stripping.

Fertilization and Hatching rates

Fertilization rate was determined as the percentage of eyed eggs following the fertilization process. Similarly, hatching rates were determined as the percentage of alevin (yolk-sac fry) following eyed ova stage (Bozkurt, 2006) [2]. Fertilization and hatching rates of rainbow trout in the control and treatment groups was assessed by using the following formula described by Muir and Robert (1985) [11].

$$Fertilization\ rate = \frac{number\ of\ fertilized\ eggs}{total\ number\ of\ eggs} \times 100$$

$$Hatching\ rate = \frac{number\ of\ hatched\ larvae}{number\ of\ fertilized\ eggs} \times 100$$

Results

The fertilization rate for each replicate of treatment groups and control group was calculated. In group T1, heat shocked at 26 ºC, after 15 minutes of fertilization (TAF) for 10 minutes, the observed fertilization rate was 90.393±0.37%. Similarly for group T2; heat shocked at 26 ºC for 10 minutes after 20 minutes of fertilization, the observed rate of fertilization was 89.473±1.04%. In case of group T3, heat shocked at 28 ºC, 15 minutes after fertilization for duration of 10 min. fertilization rate was observed to be 90.206 ± 0.94% while as for group T4, heat shocked at 28 ºC for duration of 10 minutes, applied after 20 minutes of fertilization, fertilization rate of 88.483±1.68% was observed. However, the fertilization rate for the control group observed was 93.16±0.41%. The highest fertilization rate among the treatment groups was observed in group T1 at 90.393±0.37% while the lowest fertilization rate of 88.483 ±1.68% was observed in group T4. It was found that the fertilization rate was higher in control group as compared to treatment groups. There was a significant difference (P<0.05) between the treatment groups and control group as shown in table 2.

The hatching rate was determined when the sac fry (alevin) hatched out of the eggs. This took another 18 to 22 days after the appearance of eyed ova stage. The hatching was observed after 34 to 38 days of stripping at a water temperature of 9.5 ºC. The hatching rate (mean ±S.E) for each replicate of treatment groups and control group was calculated. The hatching rate for group T1, T2, T3 and T4 was 79.746 ±1.04, 81.843±0.88% 72.153±1.17%, and 80.946±2.21%

respectively. The highest hatching rate among the treatment groups was observed in group T2 (81.843±0.88%) and the lowest hatching rate of 72.153± 1.17% was observed in group T3. The hatching rate was higher (84.28±0.46%) in control group as compared to treatment groups. It was observed that increasing heat shock intensity reduced the survival percentage of eggs from fertilization to hatching. However, increasing time after fertilization, hatching rates also increased. Survival rates showed significant differences (P<0.05) between treatment groups and control group as shown in table 3.

Table 2: Fertilization rates of treatment groups, control group and their replicates in rainbow trout (*O. mykiss*) after application of heat shock.

Treatment Groups	Replicates	Fertilization rate (%)	Mean ± SE	P Value
T1	T1-1	89.84	90.393±0.37	<0.05
	T1-2	91.11		
	T1-3	90.23		
T2	T2-1	87.38	89.473±1.04	
	T2-2	90.47		
	T2-3	90.57		
T3	T3-1	89.64	90.206 ± 0.94	
	T3-2	92.05		
	T3-3	88.93		
T4	T4-1	87.13	88.483 ± 1.68	
	T4-2	91.83		
	T4-3	86.49		
TC (Control)	TC-1	93.11	93.16±0.41	
	TC-2	92.47		
	TC-3	93.90		

Table 3: Hatching rates of treatment groups, control group and their replicates in rainbow trout (*O. mykiss*) after application of heat shock.

Treatment Groups	Replicates	Hatching rate (%)	Mean ± SE	P Value
T1	T1-1	78.26	79.746 ±1.04	< 0.05
	T1-2	81.76		
	T1-3	79.22		
T2	T2-1	80.72	81.843 ± 0.88	
	T2-2	83.59		
	T2-3	81.22		
T3	T3-1	74.37	72.153 ± 1.17	
	T3-2	71.70		
	T3-3	70.39		
T4	T4-1	79.66	80.946 ±2.21	
	T4-2	85.27		
	T4-3	77.91		
TC (Control)	TC-1	84.19	84.28±0.46	
	TC-2	83.53		
	TC-3	85.13		

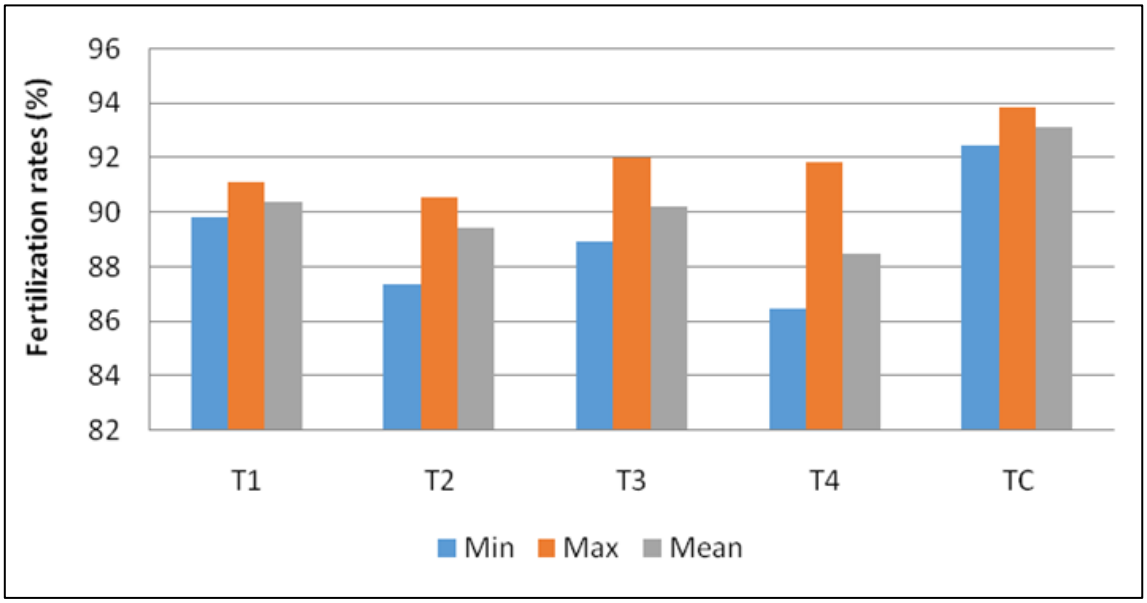

Fig 1: Minimum, maximum & mean values of fertilization rates among treatment groups and control group

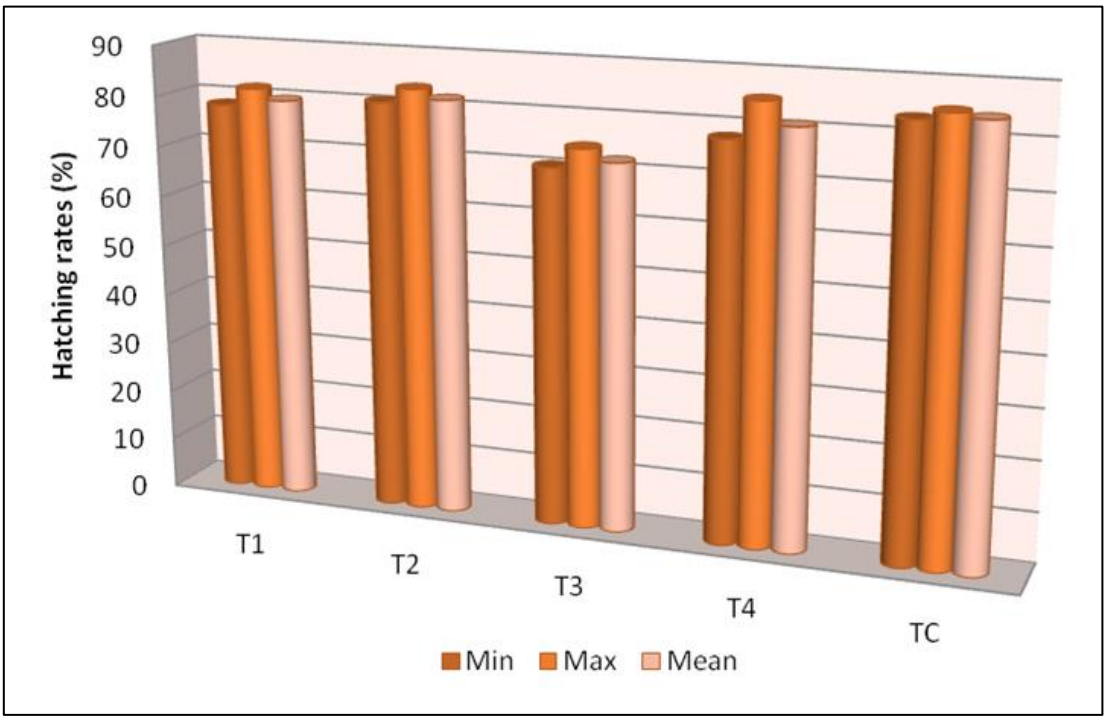

Fig 2: Minimum, maximum & mean values of hatching rates among treatment groups and control group

Plate 1: Eyed ova stage of rainbow trout (*O. mykiss*)

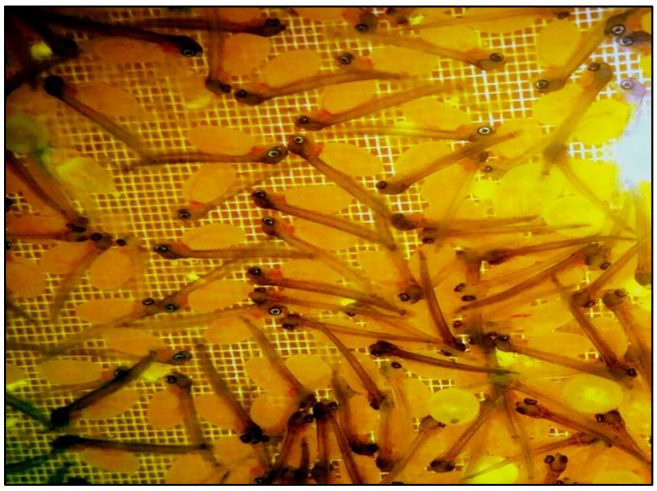

Plate 2: Newly hatched alevin (sac fry) of rainbow trout (*O. mykiss*)

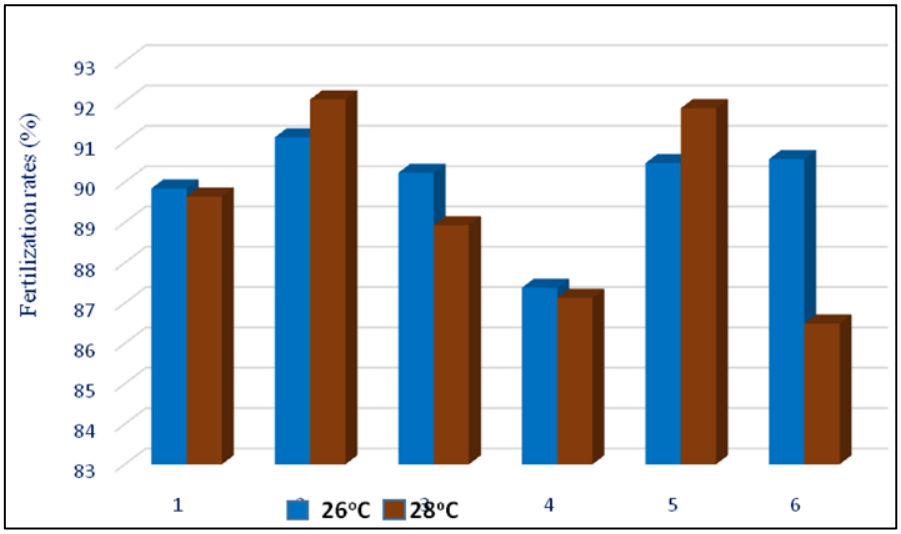

Fig 3: Graphical representation of fertilization rates (%) heat shocked at 26 °C & 28 °C.

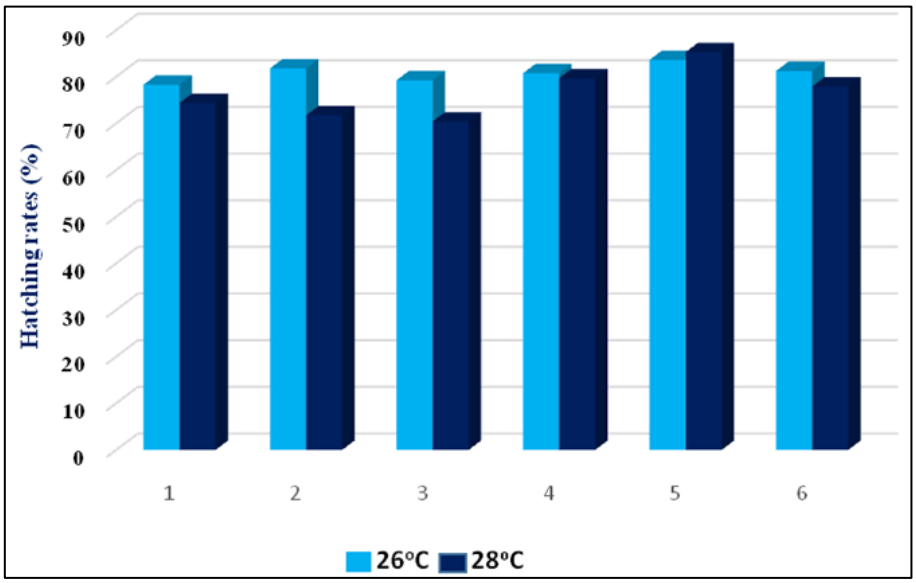

Fig 4: Graphical representation of hatching rates (%) heat shocked at 26 °C & 28 °C.

Discussion

For triploid fish production, biotechnological methods such as heat shock are frequently used. Heat shock has influenced the survival rates of rainbow trout eggs from fertilization to hatching (Ingrid *et al*., 2016) [9]. In the present study, the average fertilization rate in treatment groups was low (88.483±1.68%) compared to the control group (93.16±0.41%). Saber and Pourkazemi (2012) [16] reported similar findings, with low fertilization rates in the treatment groups and their experimental replicates and relatively high fertilization rates in the control group. Negative effects of heat shock may be one of the reasons for low fertilization (Cherfas *et al*., 1994) [6]. Chourrout (1986) [7] found that in rainbow trout, fertilized eggs subjected to heat shock for 2, 4, 6, 8, 10, 15, and 20 minutes at a temperature of 26 °C, the rate of survival was more than 90%. Quillet, 1990 [15] reported a survival rate of 66-89% in *Salmo salar* eggs exposed to shocks at temperatures ranging from 26 to 29 °C for 15 and 10 minutes after fertilization. Aruljothi, 2015 [1] obtained maximum fertilization rate (91.4%) at 38 ºC for duration of 1 min after fertilization (TAF). Bazaz, 2019 [3] reported that heat shock at 30 ºC, 12 min. after fertilization (TAF) for duration of 10 min. gave maximum fertilization percentage of 76.67±1.24%. However, fertilization rates were higher in control group then treatment groups.

In this study, the control group had a higher hatching rate than the treatment group. Heat shock 20 minutes after fertilisation (MAF) at 26 °C produced the highest hatching rate (81.843± 0.88%) among the treatment groups, while heat shock 15 minutes after fertilization (MAF) at 28 °C produced the lowest hatches (72.153±1.17%). The control group had the highest hatching rate of all, at 84.28 ±0.46%. Similar results have been reported by many authors. Dillon (1988) [8] reported that eggs heat-shocked at 28 °C had slightly lower hatch rates ($p< 0.01$) than eggs heat-shocked at 26 °C. The author recorded that eggs heat-shocked 10 minutes after fertilisation (MAF) at 26 °C had significantly lower hatch rates than eggs heat-shocked 20, 30, or 40 MAF, and that eggs heat-shocked 10 or 20 MAF at 28 °C had significantly lower hatch rates than eggs heat-shocked 30 or 40 MAF. Similarly, in the present study, the eggs heat shocked at 15 min after fertilization (MAF) at 26 °C had significantly lower percent hatches than eggs heat-shocked 20 MAF at same temperature, while as eggs heat-shocked 15 MAF at 28ºC had significantly lower hatches than those with heat shocks initiated 20 MAF. The decreased hatching rates in treated lots were due primarily to the heat shocks themselves (Dillon, 1988) [8]. Lower percent hatch in heat shocked eggs compared to controls have been previously reported by many workers (Thorgaard *et al*., 1981; Scheerer and Thorgaard 1983; Solar *et al*., 1984) [21, 17, 19]. Highest hatching rate of 87.2% was recorded by Aruljothi, 2015 [1] at 38 ºC for duration of 1 min TAF. Bazaz, 2019 [3] reported that heat shock initiated at 10 min. after fertilization resulted in hatching rates of (58.20±1.73%). However, hatching rates were relatively higher at 12 minutes post fertilization (64.32±0.94%). The author observed that by increasing the shock time after fertilization (TAF), the hatching rates also increased considerably. These results are in harmony with present study.

Conclusion

This study is beneficial to fish farmers with respect to culture practices of rainbow trout (*O. mykiss*) in terms of development and growth. The demand of rainbow trout in Kashmir valley is high as compared to supply; this study may be helpful in increasing production levels with improved survival. The present study endaevour towards creating a bridge between supply and demand of rainbow trout, to meet the required demand of production. This study can serve as a basis to investigate further shock protocols, with respect to maximum survival rates. Quality seed production is an important objective in aquaculture; this study will be useful to fish farmers with respect to artificial seed production, and will in turn reduce the input cost.

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