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Influence of Thermal Shock Intensity on the Early Developmental Stages of Eggs in Cold Water Salmonid species, Brown Trout (*Salmo trutta fario*), in the Hatcheries of Kashmir Himalayas

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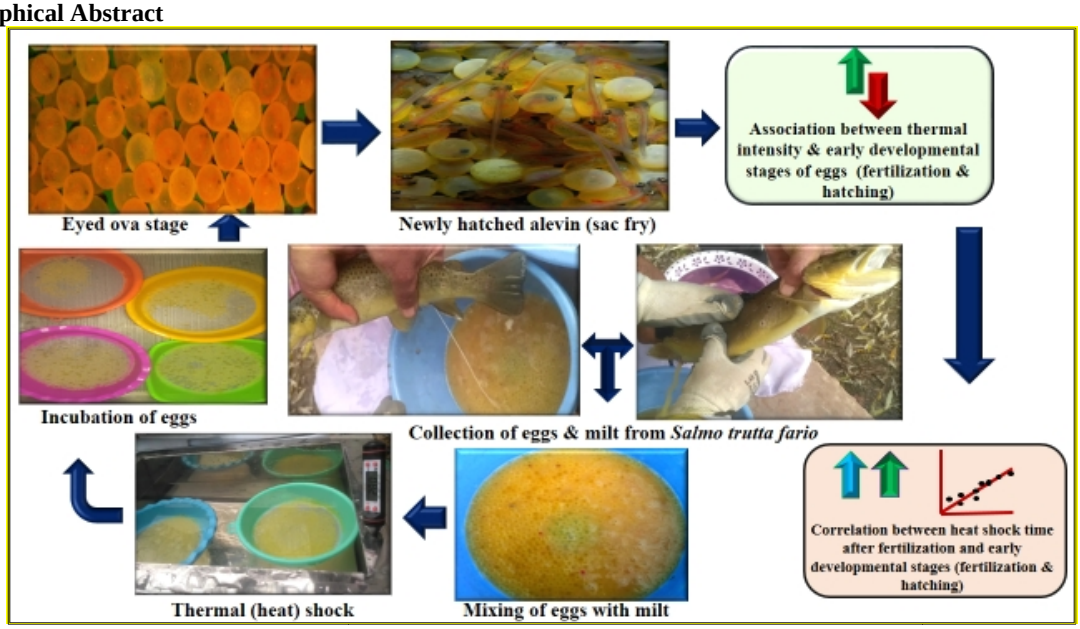
ABSTRACT: Brown trout (*Salmo trutta fario*) is an important fish species for recreational fishing because of its aquaculture potential, economic worth, and widespread consumer demand. In aquaculture, the primary focus in thermal induced triploidization is the production of sterile fish which prevent negative effects on growth and survival related to the gonadal maturation. The present study was conducted in government trout farm Laribal, Srinagar to observe effects of various heat shock treatments on the fertilization and hatching rates of eggs in brown trout (*S. trutta fario*). Four heat shock treatments and a control group in three replicates were observed. The highest fertilization rate among the treatment groups was observed in group T2 (26°C, 20 minutes after fertilization) with 87.55±0.54%. There was a significant difference (P<0.05) between the treatment groups and control group. It was found that the fertilization rate was higher in control group (92.32±0.51%) as compared to treatment groups. Similarly the hatching rate was higher (80.16±0.64%) in control group as compared to treatment groups. The highest hatching rate among the treatment groups was observed in group T2 (78.72±1.30%) at 26°C, 20 minutes after fertilization. Hatching rates showed significant differences (P<0.05) between treatment groups and control group. Temperature intensity formed significant negative correlation between fertilization rate and hatching rate (r = -0.725, p<0.01; r = -0.909, p<0.01) respectively. While as, fertilization rates and hatching rates showed significant positive correlation with heat shock time after fertilization (r = 0.5711, p<0.01; r = 0.375, p<0.01) respectively. The present study revealed that the heat shock treatment had a significant influence on the early survival rates of eggs in brown trout.

Keywords: (*Salmo trutta fario*), Heat shock, Eggs, Fertilization, Hatching.

Graphical Abstract



INTRODUCTION

Brown trout (*Salmo trutta fario)*, is a cold-water fish belonging to family Salmonidae. The Salmonid species (*S. trutta fario*) are native to Europe, North America, Africa, Australia, New Zealand and Papua New Guinea (Moyle 2002). Brown trout (*Salmo trutta fario*) is an important fish species for recreational fishing because of its aquaculture potential, economic worth, and widespread consumer demand. In general, triploids are considered to be sterile, because homologous chromosomes are likely to fail properly during meiosis. In aquaculture, the primary focus in triploidization is the production of sterile fish which prevent negative effects on growth, survival and meat quality related to the gonadal maturation. A variety of techniques have been developed to induce triploidy in fish (Thorgaard, 1983). Thermal shock such as cold shock and heat shock treatments (Sun *et al*., 1992; Yang *et al*., 1997; Pandian and Koteeswaran, 1998) are considered safe for triploid induction as no harmful chemicals are used. Heat shock is an efficient and commonly used method for inducing polyploidy in fish. Nevertheless, it is not always successful and can have negative consequences (Bazaz *et al*., 2020). The most successful triploid production in salmonids was achieved by applying hydrostatic pressure to newly inseminated and activated eggs (Arai, 1984, 1987; Chourrout, 1984; Lou and Purdom, 1984). However, due of the requirement for specialised equipment and the difficulties in treating a large number of eggs, the approach is not widely used. Scheerer and Thorgaard (1983) effectively produced triploidy in brown trout using heat shocks. Thermal shock treatment is usually preferred due to the convenience of the operation and the simplicity of the equipment utilised. Keeping in view the above mentioned benefits, the current study aimed to examine the effects on the early developmental stages of eggs in brown trout exposed to various thermal shock treatments in view of its importance in inducing triploidy for aquaculture benefits.

MATERIALS AND METHODS

A. Brood-stock collection and segregation

Male and female brooders of brown trout (*Salmo trutta fario*) have been acquired from the Trout Culture Farm, Laribal, Srinagar (J&K Govt.). The parental brood stock was fasted for 48 hours prior to sperm and egg collection during the pre-spawning season. The length of female brown trout ranged from 32.3cm to 45.4cm with a mean value of 37.98 ± 1.30cm while as for male brown trout, the length ranged from 32.3 cm to 45.4cm with a mean value of 37.98 ± 1.38 cm. The observed weight of female brown trout ranged from 615g to 1137g with a mean value of 757.6 ± 57.22g while as the male brown trout weighed in the range of 613g to 975g with a mean value of 772.7 ± 41.4g. The spawning fecundity per female ranged from 961 to 1604 eggs and the mean spawning fecundity of 1124.8 ± 71.60 eggs was observed.

B. Stripping and fertilization

As outlined by Bozkurt (2006), the fertilization process was carried out using the dry stripping method in brown trout (*Salmo trutta fario*). 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.

The eggs were divided into 4 treatment groups (replicated thrice) and a control group (Table 1). Heat shock was given at 26°C after 15 minutes after fertilization for 10 minutes, to the first trial unit (T1), for another trial unit (T2), heat shock was set at 26°C after 20 minutes of fertilization for 10 minutes. While as, trial unit (T3) heat shock at 28°C was given after 15 minutes of fertilization for 10 minutes and last trial unit (T4) heat shock at 28°C was given after 20 minutes of fertilization for 10 minutes to induce triploidy. For each temperature treatment, the eggs were kept in a hot water bath. The temperature of water bath was maintained and continuously checked with an infrared thermometer.

Table 1: Heat shock treatment details in *Salmo trutta fario*.

Groups	Recurrence number	Treatment	Shock Duration (Min)	Time after Fertilization (TAF)(min)	Shock Temperature
T1	**I, II, III**	Heat	10	15	26°C
T2	**I, II, III**	Heat	10	20	26°C
T3	**I, II, III**	Heat	10	15	28°C
T4	**I, II, III**	Heat	10	20	28°C
Control (TC)	**I, II, III**	—	—	—	—

C. Incubation of Eggs

Following heat shock procedure eggs were carefully placed in perforated hatching trays maintained with a continuous supply of freshwater for incubation. The water temperature was regularly monitored during the incubation period.

D. Fertilization and Hatching rates

Fertilization rate was estimated as the percent of eyed ova following the fertilization process. Hatching rates were calculated in the same way, as a percentage of alevin (sac fry) following the eyed ova stage (Bozkurt, 2006).

Fertilization and hatching rates of brown trout in the control and treatment groups was assessed by using the following formula described by Muir and Robert (1985).

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

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

RESULTS AND DISCUSSION

Fertilization and Hatching rates were determined for 4 treatment groups (T1, T2, T3 and T4) replicated 3 times and a control group. Dead eggs were removed and counted on daily basis. The temperature of the water was monitored on a regular basis and ranged from 8.3°C to 11.2°C. For each replicate of the treatment and control groups, the fertilization rate was measured at the eyed ova stage (Plate 1).

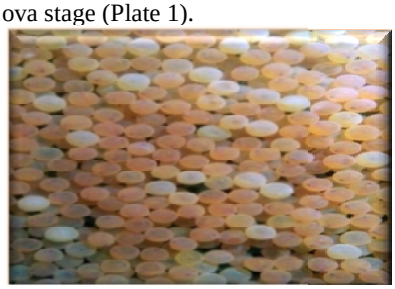


Plate 1: Eyed ova stage of brown trout (*Salmo trutta fario*).

A. Fertilization rates

The fertilization rate for each replicate of treatment groups and control was calculated (Table 2). Group T1, heat shocked at 26°C, after 15 minutes of fertilization (TAF) for 10 minutes the observed fertilization rate was 83.76±0.21%. Similarly group T2; heat shocked at 26°C for 10 minutes after 20 minutes of fertilization, the observed rate of fertilization was 87.55±0.54%. While as, in the treatment group T3, heat shocked at 28°C, for 10 minutes, 15 minutes post fertilization, the fertilization rate was observed 64.91 ± 0.90%. However, T4 group, heat shocked at 28°C for duration of 10 minutes, applied after 20 minutes of fertilization, the fertilization rate of 81.06 ± 1.46% was observed. The observed fertilization rate for the control group was 92.32±0.51%. The highest fertilization rate among the treatment groups was observed in group T2 with 87.55±0.54% and the lowest fertilization rate of 64.91 ± 0.90% was observed in group T3. The results from the present study revealed that the fertilization rate in the control group was greater than in the treatment groups. There was a significant difference ($P<0.05$) between the treatment groups and control group as shown in Table 2.

B. Hatching rates

The hatching percentage was determined when the sac fry (alevin) hatched out of the eggs (Plate 2). The hatching was observed after 50 to 56 days of stripping at a water temperature of 9°C. 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 63.08±1.52, 78.72±1.30%, 38.59±1.23%, and 46.41±1.37% respectively (Table 3).

Table 2: Fertilization rates of treatment groups, control group and their replicates of brown trout (*Salmo trutta fario*) after application of heat shock.

Treatment Groups	Replicates	Fertilization rate (%)	Mean ± SE	P Value
T1	T1-1	84.02	83.76±0.21	<0.05
	T1-2	83.93		
	T1-3	83.33		
T2	T2-1	88.49	87.55±0.54	
	T2-2	87.58		
	T2-3	86.59		
T3	T3-1	63.23	64.91 ± 0.90	
	T3-2	65.16		
	T3-3	66.35		
T4	T4-1	83.94	81.06 ± 1.46	
	T4-2	80.12		
	T4-3	79.12		
TC (Control group)	TC-1	91.43	92.32±0.51	
	TC-2	92.33		
	TC-3	93.22		

The highest hatching rate among the treatment groups was observed in group T2 (78.72±1.30%) and the lowest hatching rate of 38.59±1.23% was observed in group T3. The hatching rate was higher (80.16±0.64%) in control group as compared to treatment groups. Hatching rates showed significant differences ($P<0.05$) between treatment groups and control group as shown in Table 3. The Pearson correlation between fertilization rate, hatching rate with heat shock temperature intensity and heat shock time after fertilization is given in Table 4.

Plate 2: Newly hatched, alevin (sac fry) of brown trout (*Salmo trutta fario*).

Table 3: Hatching rates of treatment groups, control group and their replicates of brown trout (*Salmo trutta fario*) after application of heat shock.

Treatment Groups	Replicates	Hatching rate (%)	Mean ± SE	P Value
T1	T1-1	60.33	63.08 ±1.52	< 0.05
	T1-2	63.32		
	T1-3	65.61		
T2	T2-1	78.20	78.72 ± 1.30	
	T2-2	81.19		
	T2-3	76.77		
T3	T3-1	38.58	38.59 ± 1.23	
	T3-2	40.75		
	T3-3	36.46		
T4	T4-1	46.78	46.41 ±1.37	
	T4-2	48.57		
	T4-3	43.88		
TC (Control group)	TC-1	81.26	80.16±0.64	
	TC-2	79.04		
	TC-3	80.20		

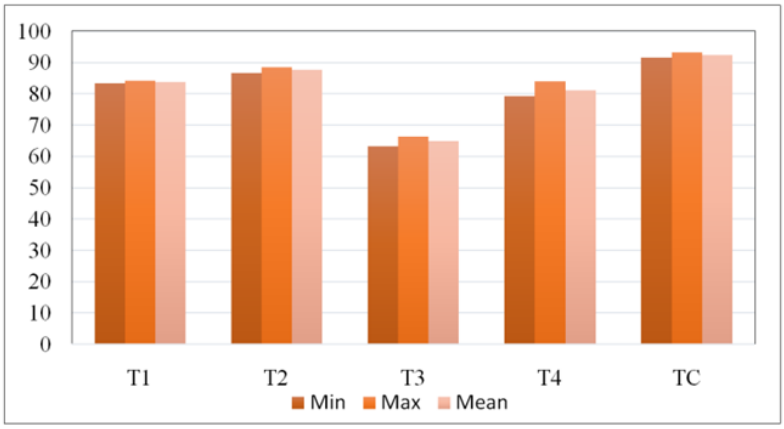


Fig. 1. Minimum, maximum and mean values of fertilization rates among treatment groups and control group.

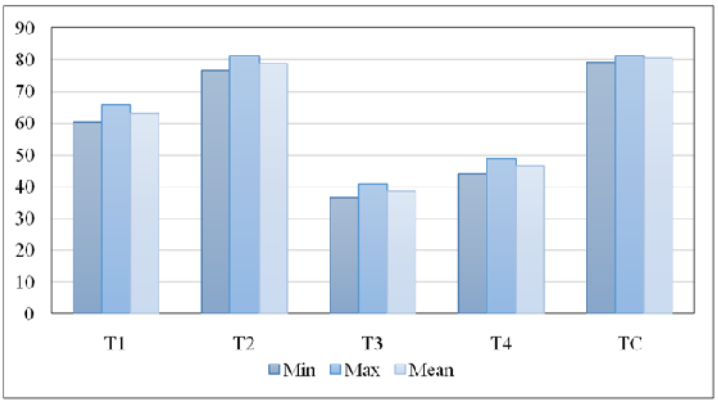


Fig. 2. Minimum, maximum and mean values of hatching rates among treatment groups and control group.

Temperature intensityshowed significant negative correlation between fertilization rate (Fig. 5) and hatching rate (Fig. 6) (r = -0.725, p<0.01; r = -0.909, p<0.01) respectively. While as, fertilization rates (Fig. 3) and hatching rates (Fig. 4) showed significant positive correlation with heat shock time after fertilization (r=0.5711, p<0.01; r= 0.375, p<0.01) respectively.

Table 4: Pears on correlation between fertilization rate, hatching rate, temperature intensity and heat shock time after fertilization.

	Fertilization rate	Hatching rate
Temperature intensity	-0.725*	-0.909*
Heat shock time after fertilization	0.5711*	0.375*

*Correlation is significant at 0.01 level

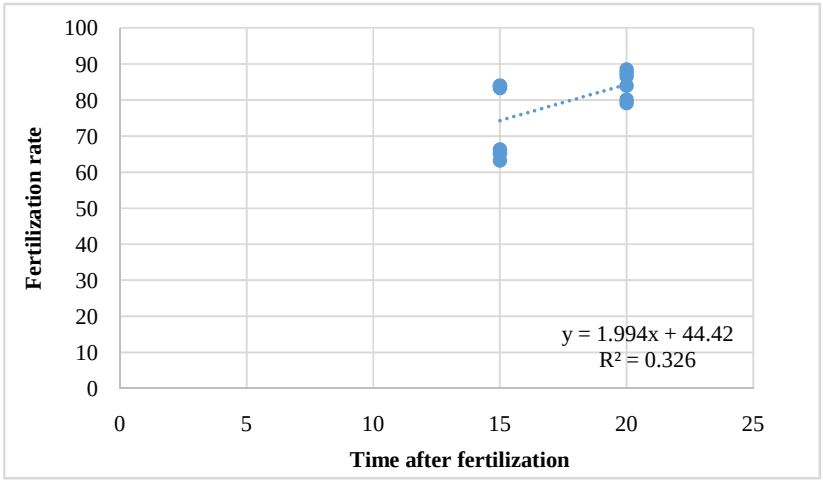


Fig. 3. Scatter plot showing relationship between fertilization rates and heat shock time after fertilization (15 minutes & 20 minutes).

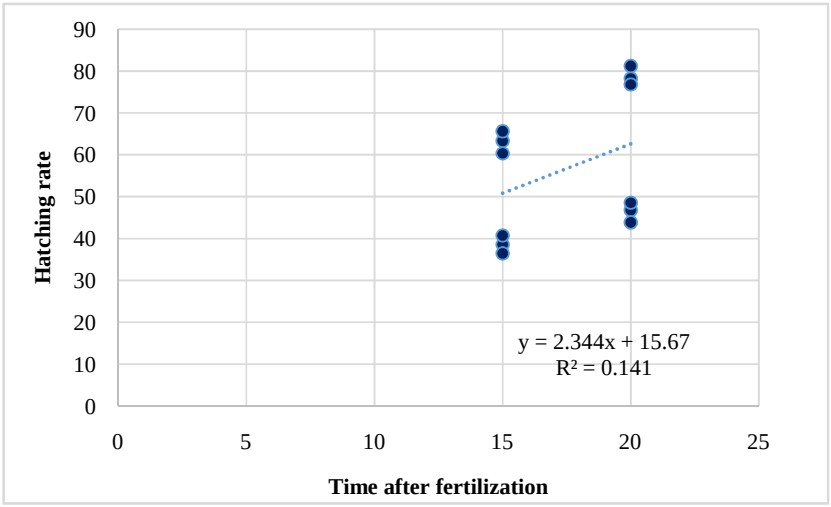


Fig. 4. Scatter plot showing relationship between hatching rates and heat shock time after fertilization (15 minutes & 20 minutes).

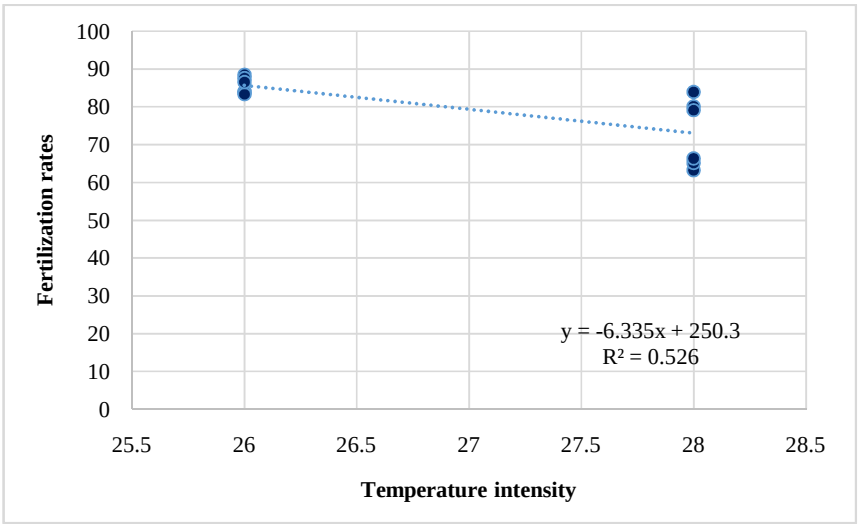


Fig. 5. Scatter plot showing relationship between fertilization rates and heat shock temperature intensity (26°C & 28°C).

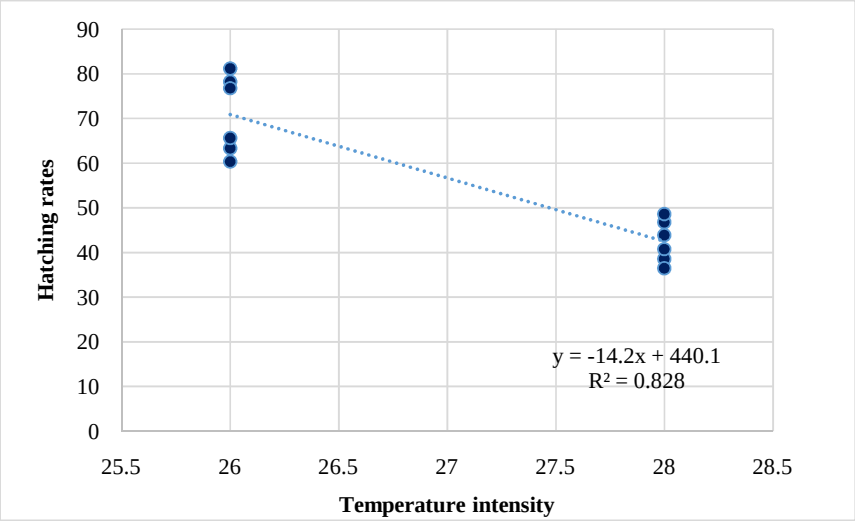


Fig. 6. Scatter plot showing relationship between hatching rates and heat shock temperature intensity (26°C & 28°C).

DISCUSSION

In the present study, the fertilization rates ranged from 64.91± 0.90% to 87.55±0.54% in treatment groups T3 and T2 respectively. The fertilization rate was even higher in control group (92.32±0.51%) compared to all treated groups. Highest fertilization rate was obtained in group T2 at 26°C for the duration of 10 minutes, 20 min. time after fertilization. However, lowest rate was obtained in group T3 at 28°C for duration of 10 minutes, 15 min. time after fertilization. Heat-shock initiated at 2 and 3 minutes after fertilization resulted in hatching rates of less than 50%, (Pradeep *et al*., 2012). However, at 4, 5, and 6 minutes after fertilization, the hatching percentages were relatively higher as 62.7%, 66.9%, and 69.8 percent, respectively. Diaz *et al*., (1993) reported that 26.5°C heat-shock application for 15 min at 15 and 25 min after fertilization resulted in the survival rates in eyed-stage that ranged 46 % and 93 %. Similarly, Dogankaya and Bekcan (2014) found that after applying a 28°C heat shock for 10 minutes at 10, 15, and 20 minutes after fertilization, the survival rate in the eyed stage was as 44.93± 1.2 %, 51.19±1.2 % and 40.02±0.3%. These results are in conformity with present study. Bazaz, (2019) reported that heat shock at 30°C, 12 min TAF for duration of 10 min. gave maximum fertilization percentage of 76.67±1.24%.

The present study demonstrated that heat shock administration to brown trout eggs at 26°C, for duration of 10 minutes, 20 min. time after fertilization (TAF) resulted in effective hatching rates (78.72± 1.30%) as compared at 28°C, for duration of 10 minutes after 15 min.time after fertilization (TAF) which were comparatively less (38.59± 1.23%). More promising results with around 79% at hatching were obtained as compared to the other studies, from which the best survival rates reported by Scheerer and Thorgaard (1983) and Arai and Wilkins (1987) were 44% and 57% respectively. The difference may possibly be related to the egg quality (Lou and Purdom, 1984) though survival rates in control groups were usually higher (80.31±0.62%) as compared to treatment groups. Longer shock temperatures have previously been shown to have no detrimental effects on survival rates in brook trout and brown trout (Quillet *et al*., 1991; Dube *et al*., 1991). According to Moffett and Crozier (1995), Karatas (2009), Bazaz *et al*., (2021) higher shock levels resulted in reduced egg survival rates. Solar *et al*., (1984) and Lou and Purdom (1984) conducted another study, 28°C heat-shock application for 10 minutes at 40 min after fertilization resulted in hatching larvae ratio that ranged 24 % -74%. Dogankaya and Bekcan, 2014 reported 28°C heat-shock applications in rainbow trout for 10 min at 10 and 15 minutes after fertilization, hatching larvae ratio was determined as 33.67± 1.9% and 41.02 ± 1.9 % respectively. These results are in proximity with present research. According to Bazaz, 2019, thermal shock begun 10 minutes after fertilization resulted in low hatching rates (58.20±1.73%) whereas hatching rates were substantially higher after 12 minutes of post fertilization (64.32±0.94%). These results are in consensus with present study.

CONCLUSION

The present study revealed that the heat shock treatment had a significant influence on the development of eggs in brown trout. Fertilization and hatching rates were observed to be significantly higher in control groups compared to the treatment groups. Furthermore, it was observed that increasing the intensity of the heat shock lowered the fertilization and hatching rates. However, the fertilization and hatching percentage continued to rise when the duration of shock initiation after fertilization was increased.

FUTURE SCOPE

This current research is advantageous to fish farmers with regard to culture activities of brown trout in terms of development and growth. The current study will endeavour to serve as a bridge between the supply and demand for brown trout in order to satisfy the needed production level. The present study serves as a base to further test other shock protocols with respect to maximum survival rates in brown trout. Further more

the present study suggests to further investigate the differences between heat shocked and control reared brown trout for observing various biological, biotechnological, hematological and histo-pathological parameters.

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Conflict of interest. The authors of the current study declares no conflict of interest.

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