The Effects of Pressure and Heat Shocks Induced at Different Times After Fertilization on Triploid Production in Rainbow Trout (*Oncorhynchus mykiss*, W. 1792)

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Abstract

The objective of this study was to identify the survival and triploidy rates in rainbow trout under pressure and heat shocks induced at different times after fertilization. Fertilized eggs were exposed to a heat shock of 28°C for 10 minutes at 10, 15 and 20 minutes after fertilization. Survival rates were higher at 15 min and triploidy rates were higher at 20 min applications. Also pressure shock of 48263 kPa (7,000 psi) was applied to eggs for 4 min at 35, 40 and 45 min after fertilization, and higher survival and triploidy rates were observed at 40 min treatment. These results also show that pressure shock is more advantageous comparing with heat shock in all stages of development (embryo, eyed-stage, hatching, and first feeding) in both respect of survival and triploidy rates.

Key words: *Oncorhynchus mykiss*, triploidy, heat shock, pressure shock

Döllenmeden Sonra Farklı Zamanlarda Uygulanan, Basınç ve Sıcaklık Şoklarının Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss, W. 1792*) Triploid Üretimine Etkileri

Özet

Bu çalışmanın amacı, gökkuşağı alabalığı (Oncorhynchus mykiss, W. 1792) yumurtalarına döllenmeden sonra, farklı zamanlarda basınç ve sıcak şok uygulaması yapılarak farklı dönemlerdeki yaşama oranları ile triploid oranları karşılaştırılmıştır. Yumurtaların döllenmesini takiben 10, 15 ve 20. dakikalarda 10 dakika süreyle 28°C'lik sıcak şok uygulandı. Yaşama oranı en yüksek döllenmeden sonraki 15. dakikada, en yüksek triploid oranı ise 20. dakikada yapılan şok uygulamasında elde edilmiştir. Döllenmeden sonraki 35, 40 ve 45. dakikalarda 4 dakika süreyle 7,000 psi basınç şoku uygulanan deneme grubunda en yüksek yaşama oranı ve en yüksek triploid oranı 40 dakikada basınç şoku uygulanan grupta saptanmıştır. Bu sonuçlar bütün gelişme safhalarında (embriyo, göz lekeli, kuluçka ve ilk beslenme) basınç şokunun sıcak şok ile karşılaştırıldığında daha avantajlı olduğunu göstermektedir.

Anahtar kelimeler: *Oncorhynchus mykiss*, triploid, sıcak şok, basınç şoku

INTRODUCTION

The inhibition of maturation in aquaculture has great importance from commercial point of view and has been studied in detail. The sterility of fish prevents the degradation in carcass quality and turn the energy used for maturation towards growth. Triploid fish are sterile but triploid males may exhibit secondary maturation characteristics and may produce aneuploid spermatozoa (Thorgaard 1992).

Purdom (1993) reported that triploid salmons might be sterile and desirable in aquaculture and fish management programs. The primary advantage of chromosome manipulations is the use of metabolic energy for growth instead of gonad development (Utter et al. 1983). As reported by Thorgaard and Gall (1979), sterility of triploid rainbow trout has been determined in the studies of spontaneously-occurring triploids (Thorgaard 1992). Different thermal and pressure shock protocols were applied to produce triploid

and tetraploid fish by inducing the retention of second polar body or disrupting the first cleavage division (Atay et al. 2000a; Atay et al. 2000b; Chourrout 1980; Lou and Purdom 1984; Thorgaard et al. 1981). Rainbow trout, as other fish species, are tolerant to chromosome manipulations in early developmental stages (Purdom 1993, Thorgaard 1986, 1992). Triploid and tetraploid fish may survive and have interesting characteristics in reproduction and basic genetic researches. Reproductive characteristics of triploid female rainbow trout are the main reason of the interest on these fish (Lincoln and Scott 1984).

Triploid females have a residual gonad development and juvenile levels of sex hormones. While normal diploids reach sexual maturation, triploid females maintain appearance and meat quality (Bye and Lincoln 1986). Triploid males show considerable gonad development. They develop secondary sexual characteristics and produce sperm with aneuploid chromosome sets. Consequently, all-female triploids are the most desireable ones (Lincoln and Scott 1983).

In this context, the purpose of our study was to compare survival and triploidy rates in different application time of heat shock and pressure shock and also to determine which one of these methods is more advantageous for creating triploids in rainbow trout.

MATERIAL AND METHODS

Eggs from 3-6 age groups of rainbow trout were stripped by applying external abdominal pressure and fertilized with milt taken from male rainbow trout at 10±1°C. Fertilized eggs were divided in two groups for each shock treatment. Also control groups were designed for both shock protocols (Table 1).

Table 1. Experimental design

Groups	Recurrence Number	Treatment	Shock Degree	Time After Fertilization (min)	Shock Duration (min)
S1	I II	heat	28 °C	10	10
S2	I II	heat	28 °C	15	10
S3	I II	heat	28 °C	20	10
Control (SK)	I II	—	—	—	—
B1	I II	pressure	7,000 psi	35	4
B2	I II	pressure	7,000 psi	40	4
B3	I II	pressure	7,000 psi	45	4
Control (BK)	I II	—	—	—	—

Eggs were exposed to 28±0.1°C heat shock for 10 min after 10, 15 and 20 min of fertilization. Heat shocks were applied to eggs placed in a deep net submerged into a thermo regulated aquarium.

Early pressure shocks of 7,000 psi were applied by using a hydrostatic unit (Fig. 1) for 4 min at 35, 40 and 45 min after fertilization.

Fig.1. Hydraulic pressure unit.

After shock treatments, eggs were transferred to trays containing 7 divisions and inserted in fiberglass tanks bounded to a recirculation system for incubation. During incubation period, water temperature was regulated to 12±1 °C (Diaz et al. 1993).

Dead eggs were removed daily and the quantity of dead eggs (embryos in further stages) was recorded. At the end of the experiment survival rates during embryo, eyed-stage, hatching, and first feeding periods were determined.

In attempt at triploidization, the effectiveness of treatments was ascertained by chromosome analysis on hatched fry. The hatched fry were kept swimming for 3-8 h in a 0.001 % colchicine solution. After killing the fish, the kidneys and fins were removed. These parts were kept in 0.075 M KCl for 35 min for hypotonic treatment. After hypotonic treatment, the dissociated tissue were centrifuged for 10 min and fixed with 3:1 methanol-glacial acetic acid solution for 15 min and centrifuged again. The cellular suspension was dropped onto cooled (0 °C) slides and air-dried. Patterns were stained with 4% Giemsa which was prepared in a phosphate buffer at a pH of 6.8.

The best results were selected with 40X magnification and chromosomes were counted at 100X magnification.

Yield of triploidy was calculated as multiplication of survival rates in first feeding period by triploid rates (Purdom 1993).

Statistical analysis; Obtained data were analyzed by analysis of variance (ANOVA) and means were grouped by Duncan's test (P<0.05), MSTAT statistical using computer program. Regression equation was counted using Minitab statistical program (Düzgüneş et al. 1983). Survival and triploid ratios obtained from experimental groups were transformed by angular transformation before analysis of variance (Snedecor and Cochran 1967).

RESULTS

Differences between hatching times were not taken into consideration during calculations. The number of live eggs after shock ($\overline{x} \pm s_{\overline{x}}$) in heat shocked groups were 83.33±0.82% (S1), 89.94±0.24% (S2), 88.83±0.02% (S3), and 96.65±0.56% (Control) (Table 2), and in pressure shocked groups 71.39±6.92% (B1), 77.93±1.01% (B2), 72.72±5.48% (B3), and 94.65±1.34% (Control) (Table 3). The highest numbers of live eggs after shock were observed in S1 in heat shock treatment and in B2 in pressure shock treatment. The values were significantly different (P<0.01) between groups.

Survival rates of embryos were 58.72±0.79% (S1), 65.78±1.35% (S2), 58.17±1.68% (S3) and 89.36±2.06% (Control) in heat shock treatment (Table 2), and 60.70±3.02% (B1), 66.43±1.59% (B2), 60.13±3.18% (B3), and 89.48±2.28% (Control) in pressure shocked groups (Table 3). The highest survival rates in embryos were observed in S2 and B2. The values were significantly different (P<0.01) between groups.

Table 2. Survival rates in heat shock treatment

Groups	Shock Degree	Time After Fertilization (min)	Shock Duration (min)	Total Number of Eggs	The number of live eggs after shock (%)	Survival Rates			
						Embryo Stage (%)	Eyed Stage (%)	Hatching Larvae (%)	Feeding Larvae (%)
Control	–	–	–	497.00±5.30	96.65±0.56^{a*}	89.36±2.0^{a}	80.26±0.8^{a}	73.60±1.1^{a}	72.59±1.0^{a}
S1	28 °C	10	10	572.00±46.00	83.33±0.82^{c}	58.72±0.7^{b}	44.93±1.2^{c}	33.67±1.9^{c}	33.05±2.0^{c}
S2	28 °C	15	10	497.00±8.00	89.94±0.24^{b}	65.78±1.3^{b}	51.19±1.2^{b}	41.02±1.9^{b}	40.11±2.0^{b}
S3	28 °C	20	10	425.00±14.00	88.83±0.02^{b}	58.17±1.6^{b}	40.02±0.3^{c}	29.76±0.0^{c}	29.18±0.0^{c}

* The variables shown by different small letters in a same column are statistically different at P<0.01 using Duncan Test

Table 3. Survival rates in pressure shock treatment

Groups	Shock Degree (kPa)	Time After Fertilization (min)	Shock Duration (min)	Total Number of Eggs	The number of live eggs after shock (%)	Survival Rates			
						Embryo Stage (%)	Eyed Stage (%)	Hatching Larvae (%)	Feeding Larvae (%)
Control	–	–	–	498.83±8.10	94.65±1.34[a*]	89.48±2.2[a]	84.50±1.9[a]	78.74±1.2[a]	76.51±0.8[a]
B1	48263	35	4	511.00±10.00	71.39±6.92[b]	60.70±3.0[b]	53.26±3.7[b]	42.97±4.2[b]	42.48±4.1[b]
B2	48263	40	4	470.00±76.00	77.93±1.01[b]	66.43±1.5[b]	57.80±0.4[b]	47.57±5.1[b]	47.04±5.1[b]
B3	48263	45	4	501.00±24.00	72.72±5.48[b]	60.13±3.1[b]	52.75±3.2[b]	45.33±2.4[b]	44.63±2.3[b]

* The variables shown by different small letters in a same column are statistically different at P<0.01 using Duncan Test

The survival rates in eyed-stage were 44.93±1.20% (S1), 51.19±1.29% (S2), 40.02±0.38% (S3), and 80.26±0.88% (Control) in heat shock treatment (Table 2), and 53.26±3.75% (B1), 57.80±0.44% (B2), 52.75±3.22% (B3), and 84.50±1.95% (Control) in pressure shocked groups (Table 3). The highest survival rates in eyed-stage were observed in S2 and B2. The values were significantly different (P<0.01) between groups.

The survival rates of hatching larvae were 33.67±1.93% (S1), 41.02±1.95% (S2), 29.76±0.08% (S3), and 73.60±1.10% (Control) in heat shock treatment (Table 2), and 42.97±4.25% (B1), 47.57±5.18% (B2), 45.33±2.47% (B3), and 78.74±1.29% (Control) in pressure shocked groups (Table 3). The highest survival rates in eyed stage were observed in S2 and B2. The values were significantly different (P<0.01) between groups.

The survival rates of feeding larvae were 33.05±2.06% (S1), 40.11±2.07% (S2), 29.18±0.02% (S3), and 72.59±1.05% (Control) in heat shock treatment (Table 2) and 42.48±4.16% (B1), 47.04±5.16% (B2), 44.63±2.34% (B3) and 76.51±0.84% (Control) in pressure shocked groups (Table 3). The highest survival rates in feeding larvae were observed in S2 and B2. The values were significantly different (P<0.01) between groups.

Also the highest values were observed in control groups in both treatments.

Triploidy rates were determined by chromosome analysis for 10 samples for each group. The lowest triploidy of 55% was observed in the group (S1) exposed to 28±0.1°C heat shock for 10 min at 10th min after fertilization. The highest triploidy rate was 100% in the group (B2) exposed to 7,000 psi pressure shocks for 4 min at 40th min after fertilization. Triploidy rates for all treatments are shown in Table 4 and Table 5.

Table 4. Triploidy rates in heat shock treatments

Groups	Shock Degree	Time After Fertilization (min)	Shock Duration (min)	Number of Samples	Triploidy Rate (%)
Control	–	–	–	10	0
S1	28 °C	10	10	10	55.00±5.00
S2	28 °C	15	10	10	80.00±0.00
S3	28 °C	20	10	10	90.00±0.00

Table 5. Triploidy rates in pressure shock treatments

Groups	Shock Degree (kPa)	Time After Fertilization (min)	Shock Duration (min)	Number of Samples	Triploidy Rate (%)
Control	–	–	–	10	0
B1	48263	35	4	10	75.00±5.00
B2	48263	40	4	10	100.00±0.00
B3	48263	45	4	10	95.00±5.00

The highest rates were observed in 2nd groups (S2 and B2) in both shock treatments (Fig. 2) and they were higher in pressure shock treatments than the heat shocked groups.

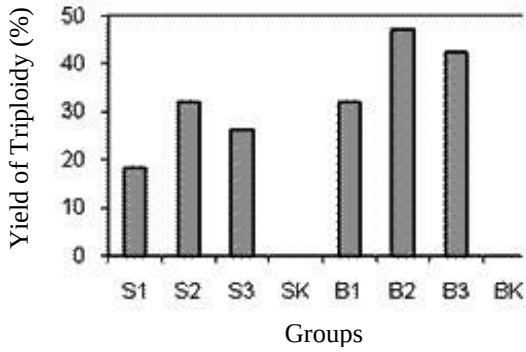

Fig. 2. The yield of triploidy in rainbow trout.

DISCUSSION

The survival ratio of 90 % in rainbow trout was obtained at 28 °C heat-shock application for 10 min at 40 min after fertilization (Lou and Purdom 1984; Solar et al. 1984). In this research at 10, 15 and 20 min after fertilization, 28 °C heat-shock for 10 min was used and percent survival ratios during embryo periods were determined as 58.72±0.7 %, 65.78 ± 1.3 % and 58.17±1.6 respectively. It was thought that differences between the means were primarily due to differences of heat-shock application times.

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 %. In our research by 28 °C heat-shock applications for 10 min at 10, 15 and 20 min after fertilization, the survival percentage in eyed stage also occurred as 44.93 ± 1.2 %, 51.19±1.2 % and 40.02±0.3 %.

Another research conducted by Lou and Purdom (1984) and Solar et al. (1984), 28 °C heat-shock application for 10 min at 40 min after fertilization resulted in hatching larvae ratio that ranged % 24 - % 74. In our research, 28 °C heat-shock applications for 10 min at 10, 15 and 20 min after fertilization, hatching larvae ratio was determined as 33.67± 1.9

%, 41.02 ± 1.9 % and 29.76 ± 0.0 % respectively. These results are in agreement with previous research.

Solar et al. (1984) notified that the survival rates of feeding larvae in groups exposed to 28 °C heat-shock for 10 min at 1 min and 40 min after fertilization, were 24 and 43 %, respectively. In our research, in groups exposed heat-shock, at 10, 15 and 20 min the survival rates of feeding larvae were 33.05± 2.0 %, 40.11±2.0 % and 29.18±0.0 % respectively. Obtained results in this research are supported with the former research.

It was observed that pressure-shock applications have been preferred commonly in research on triploid production.

One of the pressure-shock applications to triploid production was done by Lou and Purdom (1984). They reported that when 8,000 psi pressure applied for 10 min at 35 and 40 min after fertilization, embryo ratios ranged between 82 and 96 %. In this research 7,000 psi pressure applied for 4 min at 35, 40, and 45 min after fertilization and embryo ratios was found as 71.39±6.92 %, 77.93 ±1.0 % and 72.72±5.48 %.

On the research by Lou and Purdom (1984), hatching larvae rates ranged between 34 % and 80 %. In our research, larvae ratios in experiment groups were determined as 42.97±4.2 %, 47.57 ±5.1 %, and 45.33±2.4 %. These results are in harmony with that of Lou and Purdom (1984).

Shelton et al. (1986) reported that as the result of nitrous oxide with pressure application, the survival rates of feeding larvae ranged between 26 % and 78 %. In our research, as the result of pressure-shock application, the survival rates of feeding larvae determined as 42.48 ±4.1 %, 47.04±5.1 %, and 44.63±2.3. These results agree with Shelton et al. (1986).

Solar et al. (1984) notified that when rainbow trout eggs were exposed heat-shock with 24°C -30 °C changing temperature for 10 min at 1 min and 40 min after fertilization, triploid ratio ranged between 10 % and 100 %.

Lou and Purdom (1984) reported that when 28°C heat-shock applied for 10 min at 40 min after fertilization, 85-90 % triploid ones were obtained.

Diaz et al. (1993) noted that when 26.5°C heat-shock was applied for 15 min at 15 and 25 min after fertilization, triploid ratios were obtained by 73±5.6 % and 78.6±4.5 % ratios. Öztürk (1998) reported that when 28°C and 26°C heat-shock was applied for 40 min at 15 and 30 min after fertilization, 80 % and 90 % triploid ones were obtained.

In this research, 28°C heat-shock applications for 10 min at 10, 15 and 20 min after fertilization resulted in 55.00±5.00 %, 80.00±0.00 %, and 90.00±0.00 % triploid ratios, these findings agree with Diaz et al (1993), Lou and Purdom (1984), Öztürk (1998) and Solar et al (1984).

The studies aimed to obtain triploid rainbow trout through pressure-shock applications showed to provide quite high triploid ratios.

Lou and Purdom (1984) reported 8000 psi pressure application for 10 min at 40 min after fertilization resulted in 80- 90 % triploid rainbow trout.

Shelton et al. (1986) noted that when rainbow trout eggs exposed to 11 atm pressure-shock for 0-30 and 0-60 min, 90 % triploid ones were obtained.

Chourrout (1984) reported that when rainbow trout eggs were exposed to 7,000 psi pressure-shock application for 4 min at 40 min after fertilization, 100 % triploids were produced.

In this research rainbow trout eggs were exposed to 7,000 psi pressure-shock for 4 min at 35, 40 and 45 min after fertilization and this application resulted in 75±5.00 %, 100±0.00 %, and 95.00±5.00 % triploid ones at the experimental groups.

It is concluded that, pressure-shock applications after fertilization were more feasible compared to heat-shock applications with higher survival rates at eyed stage, hatching larvae, feeding larvae, and triploid ratios.

Based on the above results, it is needed that the results of research should be better looked into commercial production of fish meat of higher quality.

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