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The Survival Rate of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) at the Stages of Eyed Eggs, Larvae and Fry in Tetraploid Applications

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Abstract

This research aims to discover the effects of different pressure shocks, heat shocks and times after fertilization on the formation of tetraploid rainbow trout at the stages of eyed eggs, yolk-sac larvae, feed started fry. This research was designed using factorial completely randomized design consisting of three main treatments i.e. pressure shock as 9000 and 10000 psi, heat shock as 26.5°C and 30.5°C, time after fertilization as 300, 315 and 330 minutes. The shock duration for all treatments was 3 minutes. The survival rates of control groups were higher than the results of all other groups and significantly different. The highest survival rate in feed started fry was observed as 80.72% (9000 psi, 315 min). The following best results were obtained as 78.84% (10000 psi, 315 min), 75.57% (26.5°C, 300 min), 65.38% (30.5°C, 330 min). It is possible to conclude from these results that high pressure and heat shock levels and long time after fertilization caused to the reduction in survival rates. When maximum and minimum survival rates obtained in pressure and heat shock applications were compared, it was observed as maximum 80.72% and minimum 70.76% in pressure shock applications and maximum 75.57% and minimum 61.02% in heat shock applications. It is possible to interpret by acting from these figures that pressure shock treatments led to better results than heat shock treatments.

Keywords: Rainbow trout (*O. mykiss*), tetraploid, hydrostatic pressure shocks, heat shocks, eyed eggs, yolk-sac larvae, feed started fry

INTRODUCTION

Chromosome manipulation techniques have commonly used in fish in recent years [15,16,21,22,8,2]. These techniques are generally based on the suppression of the first cleavage division using chemical or physical methods such as heat/cold shock, pressure shock [5,20,12].

Rainbow trout was one of the species achieved successful results for developing this kind of techniques [20,6,7,8,1,18,4]. It was also reviewed that viable mature and fertile tetraploids was obtained in rainbow trout [24].

Rainbow trout, like other fish, is quite resistant to the artificial manipulation of chromosome sets in the early development stages [15,22,23]. Trout having three or four sets of chromosomes (triploid, tetraploid), can survive and in terms of basic genetic research, they have interesting properties.Tetraploid survival rate becomes lower than triploid in rainbow trout, but it reported that these fishes were produced and come to sexual maturity successfully [9]. Due to this their potential in aquaculture,they are drawing attention.Tetraploid fish has four pairs of chromosomes and may multiply. These fishes give diploid sperm [8] and eggs [9]. When mated with each other, tetraploid offspring occurs;when tetraploid fish and normal diploid fish mating,all offspring becomes triploids [8,9]. However it was found that 6% of tetraploid males permanently produced diploid sperm, while the rest produced haploid or aneuploid sperm. When mating normal females with tetraploid males releasing diploid sperm,100% triploids occurred [14].

There are also various researches conducted on the comparison of features of these fishes. It was reported that tetraploids had half the survival rate of diploids and triploids and their growth rate was 25% slower than that of diploids [8].

According to another study, tetraploid male fish had a lower fertilization capacity than normal male fish.The researchers predicted that the reason for this might probably be their greater sperm with the difficulty of the transition from the normal egg micropyle [8,3].

On the other hand, previous studies showed that triploid fish produced using the tetraploid male fish sperm better developed compared to the triploid fish produced by application of heat shock [8]. However, subsequent studies have given contrary results. While some of them supported the superiority of the triploid produced by using tetraploid male fish [13], others argued that there was no difference between them on early development stages of fishes [2].

As above mentioned, the production of tetraploids has some disadvantages such as difficulties in production, slow growth rate, low survival rate and low fertilization capacity. In spite of these drawbacks, this method is one of the most effective ways to produce sterile fish namely triploid fish.

Considering that there are few studies,it is required to conduct further researches on the production of tetraploid fish. The present study is also related to research the effects of different pressure shocks and heat shocks applied on the early life stages of tetraploid rainbow trout (*Oncorhynchus mykiss*, Walbaum). The purpose of this paper is to contribute to poliploidy studies by determining the number of dead eggs and survival rates after shock, regarding eyed egg, yolk-sac larvae and feed started fry according to various combinations of pressure and heat shocks applied.

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 8.5±1°C. Fertilized eggs were divided in two groups for each shock treatment. Eggs were exposed to 26.5±0.1°C and 30.5±0.1°C heat shock for 3 min after 300, 315 and 330 minutes of fertilization. Heat shocks were applied to eggs placed in a deep net submerged into a thermo regulated aquarium. Early pressure shocks of 9000 psi and 10000 psi were applied by using a hydrostatic unit (Fig.1) for 3 min at 300, 315 and 330 minutes after fertilization. After shock treatments, eggs were transferred to four trays containing 8 divisions and inserted in fiberglass tanks bounded to a recirculation system for incubation. During incubation period, water temperature was regulated to 8.5±1 °C.

Data obtained were analysed 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 [10]. Survival ratios obtained from experiment groups, were transformed by angular transformation before analysis of variance [19].

Figure 1. Hydraulic pressure unit.

RESULTS

The differences between hatching times were not taken into consideration during calculations. The highest number of died eggs after ($\bar{x} \pm s_{\bar{x}}$) shock were observed in D1and D2 (P>0.05) in heat shock treatment. The lowest number of died eggs after shock were observed in A1in pressure shock treatment and control1 (P>0.05) (Fig. 2, 5). The values were significantly different (P<0.05) between groups (Table 1a, 1b).

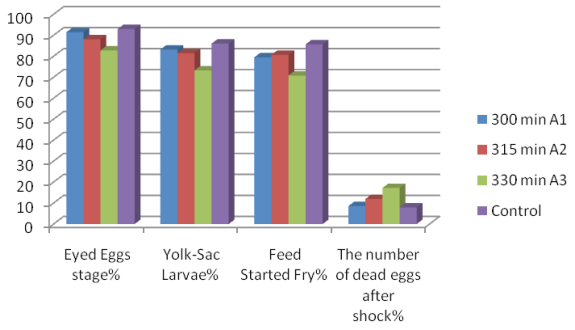

Figure 2. Survival rates in 9000 psi pressure treatment

The highest survival rates in eyed eggs stage were observed in A1in pressure shock treatment and control1 (Fig. 2). The lowest survival rates in eyed eggs stage were observed in D1 and D2 in heat shock treatment (P>0.05) (Fig. 5). The values were significantly different (P<0.05) between groups (Table 1a, 1b).

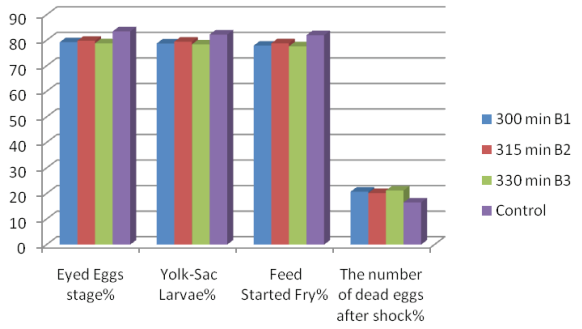

Figure 3. Survival rates in 10000 psi pressure treatment

The highest survival rates in yolk-sac larvae were observed in A1 in pressure shock treatment and control1 (Fig.2). The lowest survival rates in yolk-sac larvae were observed in D1 and D2 in heat shock treatment (P>0.05) (Fig.5). The values were significantly different (P<0.05) between groups (Table 1a, 1b).

It is possible to follow the other results obtained by applying 10000 psi pressure shock treatment and 26.5°C-30.5°C heat shock treatments from Fig. 3, Fig. 4 and Fig.5 respectively.

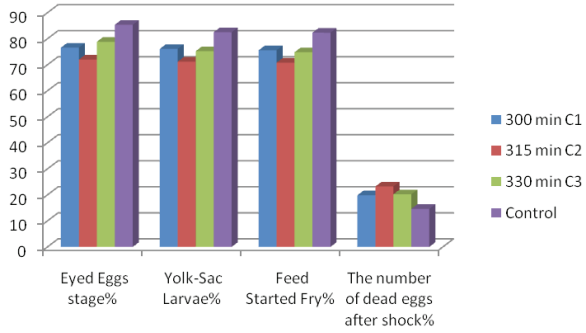

Figure 4. Survival rates in 26.5°C heat treatment

The highest survival rates in feed started fry were observed in A2 in pressure shock treatment and control1 (Fig. 2). The lowest survival rates in feed started fry were observed in D1 and D2 in heat shock treatment (P>0.05) (Fig. 5). The values were significantly different (P<0.05) between groups (Table 1a, 1b).

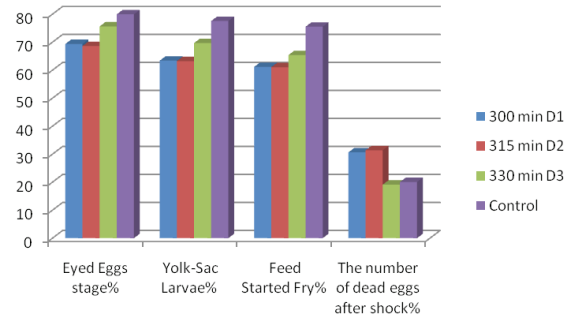

Figure 5. Survival rates in 30.5°C heat treatment

The control group, in all experimental groups in terms of eyed eggs stage, yolk-sac larvae, feed started fry and the number of dead eggs after shock received the best results (Fig. 2, 3, 4, 5).

Table 1a. Survival rates in pressure and heat shock treatment

Groups	Treatment		Time After Fertilization	Shock Duration	Total Number of Eggs	The number of dead eggs after shock		Eyed Eggs Stage	
			(min)	(min)	(number)	(number)	%	(number)	%
A1	Pressure (psi)	9000	300	3	1054.0±6.00	90.00±2.00	8.54 h	964.0±8.00	91.46 b
A2	Pressure (psi)	9000	315	3	1115.0±24.0	133.0±59.0	11.93 g	982.0±18.0	88.07 c
A3	Pressure (psi)	9000	330	3	1238.0±48.0	213.0±47.0	17.21 e	1025.0±19.0	82.79 e
Control1		-	-	-	2619.0±56.0	207.0±56.0	7.90 h	2412.0±6.00	92.10 a
B1	Pressure (psi)	10000	300	3	4273.0±7.00	886.0±20.0	20.73 c	3387.0±22.0	79.27 fg
B2	Pressure (psi)	10000	315	3	4202.0±15.0	848.0±51.0	20.18 cd	3354.0±26.0	79.82 f
B3	Pressure (psi)	10000	330	3	4261.0±42.0	902.0±30.0	21.17 c	3359.0±13.0	78.83 g
Control2		-	-	-	3955.0±22.0	653.0±37.0	16.51 e	3302.0±12.0	83.49 e
C1	Heat (^{O}C)	26,5	300	3	4052.0±75.0	805.0±12.0	19.87 cd	3100.0±32.0	76.58 h
C2	Heat (^{O}C)	26,5	315	3	4204.0±14.0	977.0±27.0	23.24 b	3027.0±34.0	72.00 ı
C3	Heat (^{O}C)	26,5	330	3	4087.0±83.0	827.0±51.0	20.23 cd	3100.0±41.0	78.85 h
Control3		-	-	-	3919.0±50.0	573.0±24.0	14.62 f	3346.0±28.0	85.38 d
D1	Heat (^{O}C)	30,5	300	3	754.0±71.0	231.0±20.0	30.64 a	523.0±38.0	69.36 j
D2	Heat (^{O}C)	30,5	315	3	857.0±29.0	269.0±23.0	31.39 a	588.0±40.0	68.61 j
D3	Heat (^{O}C)	30,5	330	3	956.0±77.0	183.0±32.0	19.14 d	723.0±31.0	75.63 h
Control4		-	-	-	3507.0±43.0	703.0±19.0	20.05 cd	2804.0±23.0	79.95 f

Table 1b. Survival rates in pressure and heat shock treatment

Groups	Treatment		Time After Fertilization	Shock Duration	Total Number of Eggs	Yolk-Sac Larvae		Feed Started Fry	
			(min)	(min)	(number)	(number)	%	(number)	%
A1	Pressure (psi)	9000	300	3	1054.0±6.00	877.0±7.00	83.21 b	838.0±43.0	79.51 d
A2	Pressure (psi)	9000	315	3	1115.0±24.0	908.0±31.0	81.44 d	900.0±31.0	80.72 c
A3	Pressure (psi)	9000	330	3	1238.0±48.0	907.0±27.0	73.26 ı	876.0±41.0	70.76 h
Control1		-	-	-	2619.0±56.0	2254.0±20.0	86.06 a	2245.0±58.0	85.72 a
B1	Pressure (psi)	10000	300	3	4273.0±7.00	3366.0±36.0	78.77 ef	3329.0±6.00	77.91 ef
B2	Pressure (psi)	10000	315	3	4202.0±15.0	3340.0±39.0	79.49 e	3313.0±19.0	78.84 de
B3	Pressure (psi)	10000	330	3	4261.0±42.0	3341.0±3.00	78.41 fg	3308.0±28.0	77.63 f
Control2		-	-	-	3955.0±22.0	3253.0±15.0	82.25 c	3244.0±31.0	82.02 b
C1	Heat (^{O}C)	26.5	300	3	4052.0±75.0	3084.0±17.0	76.11 h	3062.0±57.0	75.57 g
C2	Heat (^{O}C)	26.5	315	3	4204.0±14.0	2997.0±26.0	71.21 j	2977.0±10.0	70.81 h
C3	Heat (^{O}C)	26.5	330	3	4087.0±83.0	3075.0±30.0	75.24 h	3058.0±49.0	74.82 g
Control3		-	-	-	3919.0±50.0	3233.0±39.0	82.50 bc	3227.0±60.0	82.34 b
D1	Heat (^{O}C)	30.5	300	3	754.0±71.0	478.0±24.0	63.40 l	461.0±34.0	61.14 j
D2	Heat (^{O}C)	30.5	315	3	857.0±29.0	542.0±42.0	63.24 l	523.0±26.0	61.02 j
D3	Heat (^{O}C)	30.5	330	3	956.0±77.0	666.0±34.0	69.67 k	625.0±6.00	65.38 ı
Control4		-	-	-	3507.0±43.0	2720.0±6.00	77.56 g	2648.0±47.0	75.51 g

DISCUSSION

In this research, two different hydrostatic pressure shocks (9000 and 10000 psi) and two different heat shocks (26.5^{o}C and 30.5^{o}C) were applied on three different times after fertilization (300, 315 and 330 min) for 3 min duration to eggs fertilized by normal sperm to produce tetraploid rainbow trout (*O. mykiss*). In respect of survival rates at the early life stages (eyed eggs stage, yolk-sac larvae, feed started fry), the best results were achieved in the groups applied hydrostatic pressure shocks while the poorest results were obtained in the groups applied heat shocks. These results are compatible with the results of the researches conducted by [18,4]. According to these researches, it was observed that pressure treatments were more consistent than temperature shocks for producing both triploid and tetraploidy.

In addition Haffray et al. (2007) reported that higher mortality rates were observed at the eyed or hatching stages under heat shocks in comparison with the rates under pressure shocks. These results support our outcomes. In our study, the highest mortality were obtained under 30,5^{o}C heat shock at the eyed eggs stage while the lowest mortality rate was occurred under 9000 psi pressure shock.

On the other side, [11] reported that tetraploids had half the survival rate of diploids and triploids and their growth

rate was 25% slower than that of diploids. However in our study, under 9000 psi the survival rates of A1 groups were 91.46% at eyed eggs stages; 83.21% at yolk-sac larvae stage; 79.51% (A1) and 80,72 % (A2) at feed started fry stagewhereas in control group 92.10%, 86.06%, 85.72% in respectively. As shown, these results were much higher than that determined by [8].

As a result, the survival rates obtained under the pressure shock application at different times after the fertilization to rainbow trouts at the early stages were higher than those under heat shocks. It was determined that our results are consistent with the literature.

To achieve high survival rate and high tetraploid rate and to determine optimum values in chromosomal manipulation applications such as heat shock or pressure shock, many combinations of shock time, shock level and shock duration should be tested. We expect that the results of this study will shed light on researches to be held in order to produce tetraploid rainbow trout.

REFERENCES

[1] Bidwell, C.A., Chirsman, C.L., Libey, G. S., 1985. Polyploidy induced by heat shock in channel catfish. Aquaculture 51, 25-32.

[2] Blanc, J.M., Courrout, D., Krieg, F., 1987. Evaluation of juvenile rainbow trout survival and growth in half-sib families from diploid and tetraploid sires. Aquaculture 65,215-220.

[3] Blanc, J. M., Poisson H., Escaffre, A.M., Aguirre, Pl, Vallee, F., 1993. Inheritance of fertilizing ability in malel tetraploid rainbow trout (*Oncorhynchus mykiss).* Aquaculture, 110, 61-70

[4] Bury, D.D. (1989) Induction of polyploidy in percichthyid basses and ictalurid catfishes with hydrostatic pressure shocks. MSc thesis, Auburn University, Auburn, Alabama, USA

[5] Chourrout, D. 1980.Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson).Reprod. Nutr. Dev., 20: 727-733.

[6] Chourrout, D. 1982.Tetraploidy induced by heat shocks in the rainbow trout (*Salmo gairdneri* R.). Reprod. Nutr. Dev., 22, 569-574.

[7] Chourrout, D. 1984.Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. Aquaculture; 36 : 111-126.

[8] Chourrout, D., Chevassus B., Krieg F., Happe A., Burger G., Renard P. 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females - Potential of tetraploid fish. Theor. Appl. Genet., 72, 193-206.

[9] Chourrout, D. and Nakayama, I., 1987. Chromosome studies of progenies of tetraploid female rainbow trout. Theor. Appl. Genet., 74: 687-692.

[10] Düzgüneş, O., Kesici, T., Gürbüz, F. 1983. İstatistik Metotları I. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Ankara.

[11] Haffray, P., Pincent, C., Dupont-Nivet, M., Vandeputte, M., Merdy, O., Chavanne, H., Chatain, B., 2007. Heritabilities and G × E interactions for quality traits in the European sea bass (*Dicentrarchus labrax* L.). Aquaculture 272, S265.

[12] Lou, Y.D. and Purdom, C.E., 1984. Polyploidy induced by hydrostatic pressure in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., 25: 345-351.

[13] Myers, J.M. and Hershberger, W.K. 1991. Early growth and survival of heat-shock induced and tetraploid-derived triploid rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 96: 97-107.

[14] Nam, Y. K., Kim, D. S., 2004.Ploidy status of progeny from the crosses between tetraploid males and diploid females in mud loach (*Misgurnus mizolepis*). Aquaculture 236, 575-582.

[15] Purdom, C.E. 1983, Genetic engineering by the manipulation of cromosomes, Aquaculture, 33: 287-300, Amsterdam.

[16] Purdom C.E., Thompson D., Lou Y.D. 1985. Genetic engineering in rainbow trout, *Salmo gairdneri* Richardson, by the suppression of meiotic and mitotic metaphase. J. Fish. Biol., 27, 73-79.

[17] Purdom, C. E. 1993.Genetics and Fish Breeding. Chapman and Hall, 277, New York, USA.

[18] Rezk, M. (1988) Efficiency of heat pressure shocks for inducing tetraploidy in interspecific hybrids of cat- fish, *Ictalurus* spp. MSc thesis, Auburn University, Auburn, Alabama, USA.

[19] Snedecor, G.W., Cochran, W.G. 1967. Statistical Methods Sixth Edition The Iowa State University, Press Ames, Iowa USA, P:327.

[20] Thorgaard, G.H., Jazwin, M.E. and Stier, A.R., 1981. Polyploidy induced by heat shock in rainbow trout. Trans. Am. Fish. Soc., 110: 546-550.

[21] ThorgaardG.H., 1983. Chromosome set manipulation and sex control in fish. In : W.S. Hoar, D.J. Randall, E.M. Donaldson (ed.), Fish physiology, vol. 9, Part B, 405-434, Academic press, New York.

[22] Thorgaard G.H., 1986. Ploidy manipulation and performance. Aquaculture, 57, 57-64

[23] Thorgaard, G.H. 1992. Application of genetic technologies to rainbow trout. Aquaculture , 100: 85-97.

[24] Yoshikawa, H., Morishima, K., Fujimoto, T., Arias-Rodriguez, L., Yamaha, E., Arai, K., 2008.Ploidy manipulation using diploid sperm in the loach, *Misgurnus anguillicaudatus*: a review. J. Appl. Ichthyol. 24, 410-414.