The Rate of Tetraploidy Determination in Rainbow Trout (Oncorhynchus Mykiss) in Embryonated Eggs

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Abstract
Polyploidy at fish is characterized by modification of normal diploid chromosome set (2n) to triploid (3n), tetraploid (4n) etc. Experiments were carried out on biological material from rainbow trout (*Oncorhynchus mikiss*) during the natural period of reproduction. Polyploidy can be induced by exposing the eggs to heat shock. The highest levels of embryo losses are observed in the first two decades of incubation. The success rate of the tetrapolidy induction after 6 hours after fertilization evaluated by karyotype analysis was 66.67%. During the whole incubation period for the experimental group the losses were evaluated at 39.86% from the incubated eggs, and for the control group at 13.48%.

Keywords: heat shock, rainbow trout, tetrapoidy, kariotype analysis

1. Introduction

Polyploidy at fish is characterized by modification of normal diploid chromosome set (2n) to triploid (3n), tetraploid (4n) etc [1,2,3]. Polyploidy can be lethal to some species (mammals and birds) [4]. Polyploidy can be induced by exposing the eggs to heat shock, extreme pressure, pH, different concentrations of chemicals [4,5,6,7]. Sometimes polyploidy can appear in natural way.

Polyploid cells are having in plus one or more sets of chromosomes (3n, 4n), so they will have a bigger nucleus. Cytoplasm/nucleus rapport is constant, so that if the nucleus volume is increasing the cytoplasm volume will increase proportionally and the cell will be bigger. Theoretically triploids will have bigger cells than diploids; tetraploids will have bigger cells then triploids and diploids, etc. The increased dimension of the polyploid cells is variable at all cells and tissues.

Polyploidy can be identified by karyotype analysis (visualization and counting number of chromosomes), flow cytometry, cell measurement by Coulter Counter Channelyzer or blood smear, silver staining nuclear organization regions (6) izozymes analysis with markers [8,9]. Karyotype analysis is the most precise one, but it is a very slow and laborious method [10]. Flow cytometry is a fast methods but the equipments are very expensive [11]. Coulter Counter Channelyzer analysis is a rapid method and the equipments are cheap [11]. Cell measurement on blood smear is a precise method [8]. Nuclear organization regions (NORs) analysis is the cheapest and it can be carried out easier then karyotype analysis; this method can be applied to young fish without sacrificing them. The principle of this method is based on establishing the numbers of NORs (1 NOR per haploid genome) [9].

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2. Materials and methods

Experiments were carried out on biological material from rainbow trout (*Oncorhynchus mikiss*) during the natural period of reproduction. Eggs were collected by abdominal pressure (aprox. 4000 eggs) from two females, one three years old, respectively four years old. Sperm was collected from four two years of males and the eggs were fertilized by wet method. After fecundation the excessive quantity of sperm is rinsed with water. Heat shock was induced after 6 hours after fecundation at 28 ^{O}C for 15 minutes and then the fertilized eggs were incubated. Water temperature for incubation was 7.5-8 ^{O}C, the same temperature for water used for activation and rinsing. Eggs were incubated for 45 days, summing a temperature of 340 ^{O}C. For control group were 786 normal fertilized eggs, incubated in same condition as per experimental group.

The following method was used to determine karyotype of trout embryos for both groups: 30 embryonated eggs were taken out from each group and for 6 hours were kept in 0.005% colchicine solution to block the cell division and tho obtain chromosomes in metaphase. After 6 hours embryonated eggs were transferd in 0.7% NaCl solution and then in fixation solution (3 parts methanol 1 part acetic acid). After 30 minutes the fixation solution was replaced with fresh ones usinf 1 ml solution per one embryo. After fixation embryos were stored at 4 ^{O}C in refrigerator until the evaluation. Histological samples were prepared from embryonic fragments stained with 4% Giemsa solution, pH 7.2. Smears were evaluated under the microscope by analyzing metaphase chromosomes in minimum 10 visual fields to establish the numbers of diploid (2n) and tetraploid (4n) sets of chromosomes.

3. Results and discussion

During the incubation we have watched, counted and eliminated the dead embryos. Loss dynamics per decades can be seen in Table 1.

Table 1. Embryonic dynamics losses in manipulated eggs by tetrapolidy induction in rainbow trout

Specification	Experimental group		Control group	
	Total	%	Total	%
Initial number of eggs before fertilization	4000	100%	800	100
Number of unfertilized eggs	185	4.63	14	1.75
Initial number of fertilized eggs	3815	100	786	100
Losses in first decade	580	15.20	30	3.82
Losses in second decade	665	17.43	46	5.85
Losses in 3rd decade	227	5.95	13	1.65
Losses in 4th decade	47	1.23	14	1.78
Losses in last 5 days	2	0.05	3	0.38
Total losses	1521	39.86	106	13.48

From data presented in Table 1 can be observed that the highest level of losses for experimental and control groups are in first 2 decades. At the experimental lot from 3815 fertilized eggs during the incubation 1521 eggs (representing 39.86%) are lost because of different causes; maximum loss per day is evaluated at 152 eggs. At control group out of 786 fertilized eggs during the incubation 106 were lost, representing 13.48%; maximum loss per day is evaluated at 15 eggs.

At the experimental group the average losses were $\bar{x}=33.80\pm6.38$, and at control group $\bar{x}=2.35\pm0.38$. The losses difference between the two group is significant and is statistically covered, using *t* test at range 0.1% (***$p<0.001$). In figure 1, the evolution of losses between the two experimental groups, the highest level of losses can be seen in the first 2 decades and the smallest level in the last period of incubation. After 32 days of incubation the losses are reduced to minimum for both groups.

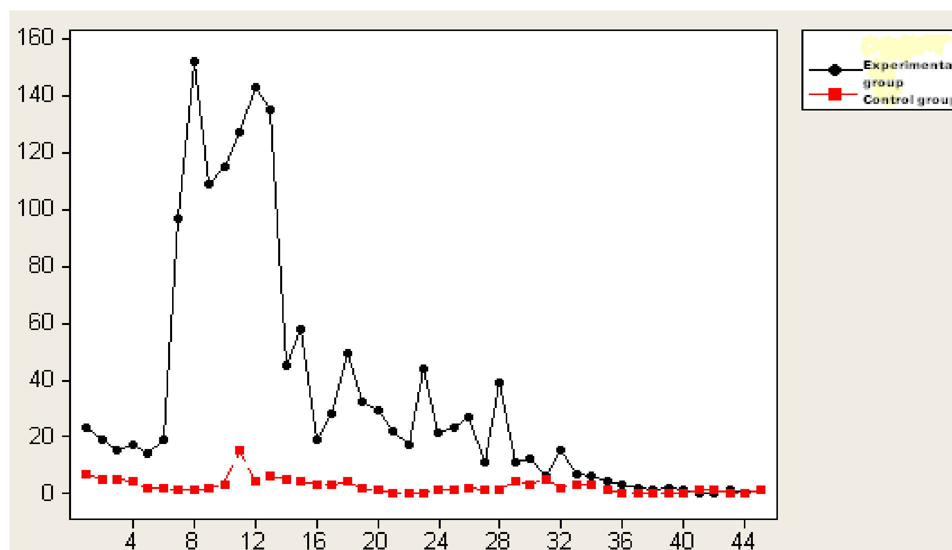

Figure 1. Loss dynamics in embryos during 45 days of incubation in the experimental group (black) and control group (red).

To establish the tetrapoidy induction rate in rainbow trout we have started from the idea that the heat shock applied after 6 hours after fertilization has the effect to block fist cell division when the diploid set of chromosomes is doubled (2n+2n=4n). In Table 2 are presented the data after karyotype analysis of embryos at the experimental and control group.

From the data that are presented in this table can be seen that in experimental group out of 30 embryos 20 are having the tertrapolid genotype, representing 66.67%; 7 embryos are having triploid genotype, representing 23.33% and 3 embryos are having diploid genotype representing 10%. In control group all embryos were having the diploid genotype.

Table 2. Results on tetrapolidy induction rate in rainbow trout eggs

Specification	Number of samples	Diploids 2n		Triploids 3n		Tetraploids 4n	
Experimental group	30	3	10.0%	7	23.33%	20	66.67%
Control group	30	30	100%	-	-	-	-

4. Conclusions

The highest levels of embryo losses are observed in the first two decades of incubation and after 32 days the losses are reduced to minimum.

During the whole incubation period for the experimental group the losses were evaluated at 39.86% from the incubated eggs, and for the control group at 13.48%.

At the experimental lot the losses are significantly higher at the range of ***p <0.001.

The success rate of the tetrapolidy induction after 6 hours after fertilization evaluated by karyotype analysis was 66.67%.

We consider that the reason of appearing of 10% diploids was the delay of heat shock induction after the first cell division, and for the 23.33% triploids the root cause was the delay in elimination of the second polar body.

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