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Antioxidant defenses during early developmental stages in tetraploid rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

Antioxidant defense status was examined during early developmental stages of rainbow trout, *Oncorhynchus mykiss*, as affected by heat shock (HS) treatment to induce tetraploidy. Samples were collected at 3850 degree-min post-fertilization at 10 °C and at eyed, hatch, swim-up, in addition to fry stages to determine the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and levels of vitamin C, A and E as well as malondialdehyde (MDA). HS treatment caused a significant reduction in survival, but not affected growth compared to the control. CAT and SOD activities peaked at hatch, whereas GPx activity reached a maximum at the swim-up stage, showing similar patterns, however, significantly higher activities in the HS group. Vitamin levels were at their minimum at the hatch in both groups, while significantly lower in the HS groups. Vitamin levels were at their highest at the fry stage, with the exception of low vitamin E in the HS group. The levels of MDA showed a steady increase during development, with consistently higher levels in the HS group. These results suggest that HS treatment and/or tetraploidy status cause high oxidative stress, as evidenced by higher activity of antioxidant enzymes and MDA levels, and lower levels of vitamins.

Keywords: Ontogenetic development; Antioxidant enzymes; Lipid peroxidation; Late heat-shock.
Article type: Research Article.

INTRODUCTION

Sexual maturation and its effects on important aquaculture production traits, such as growth and organoleptic qualities, can be reduced using sterile triploid populations of fish (Benfey 1999). However, despite these advantages, triploids are not widely used for commercial production due to the reduced performance (Fraser *et al*. 2012), as well documented for Atlantic salmon, *Salmo salar* (Benfey 2016; Madaro *et al*. 2022). Such triploids are usually produced by preventing completion of the second meiotic division shortly after fertilization (Piferrer *et al*. 2009). An alternative approach to producing triploids is via interploid crossing, usually using tetraploid sires to fertilize eggs from diploid dams (Myers *et al*. 1986; Arai & Fujimoto 2018). In rainbow trout, *Oncorhynchus mykiss*, such interploid triploids have shown superior survival and growth, while lower incidence of deformities, in comparison to the induced triploids, suggesting that it may be the treatments used for triploidy induction that are responsible for their lower performance under commercial culture conditions rather than triploidy per se (Weber *et al*. 2014). The production of tetraploids by suppression of karyokinesis or cytokinesis during zygotic cell division (Arai & Fujimoto 2018) has been achieved in several fish species, including rainbow trout (Hershberger & Hostuttler 2005; 2007), brook trout, *Salvelinus fontinalis* (Weber *et al*. 2015), yellowtail tetra, *Astyanax altiparanae* (do Nascimento *et al*. 2020), and African catfish, *Clarias gariepinus* (Okomoda *et al*. 2021). However, poor survival and performance of first-generation tetraploids, mainly during early developmental

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stages, has been an impediment to the establishment of tetraploid broodstock as the first step for breeding programs to produce triploid populations (de Alvarenga *et al*. 2020). During fish embryogenesis and larval development, de novo tissue formation and high rates of cell division are associated with an elevated metabolic rate and increased oxygen demand, resulting in the increased endogenous production of reactive oxygen species (ROS; Peters *et al*. 1994). These potentially-harmful intermediates can be responsible for oxygen toxicity and oxidative stress, mainly during early ontogeny (Davies 1995), and, as a consequence, DNA damage, enzyme oxidation, lipid peroxidation, and apoptosis (Peters & Livingstone 1996). High tissue levels of glucose and highly unsaturated fatty acids that are needed to support the energy requirements of developing fish make them more vulnerable to ROS production (Fridovich 1998). Subsequent oxidative stress can result in growth reduction, liver and muscle degradation, and death (Fontagné *et al*. 2008). In animals, ROS and other free radicals are continuously regulated by the antioxidant defense system to maintain health and prevent mortality. Such enzymatic and non-enzymatic defense systems are present in fish (Martinez-Alvarez *et al*. 2005) and shellfish (Barim-Oz & Yilmaz 2017) including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as low molecular weight vitamins such as vitamin E (Ahmad 1995). In addition, vitamin A, β-carotene, ubiquinol, and astaxanthin are also reported to have antioxidant activity in fish (Palacios *et al*. 1996). The ontogenetic development of these antioxidant defense systems has been studied in a number of fish species including Adriatic sturgeon, *Acipenser naccarii* (Díaz *et al*. 2010), turbot, *Scophthalmus maximus* (Peters & Livingstone 1996), rainbow trout (Fontagné *et al*. 2008; Zengin *et al*. 2015) and greater amberjack, *Seriola dumerili* (Pérez *et al*. 2020). The efficacy of these defense mechanisms is affected by environmental stress such as pollution (Zahmatkesh *et al*. 2020; Mohammad *et al*. 2021; Farabi *et al*. 2022; Hussein *et al*. 2022; Imanpour Namin *et al*. 2022), temperature or chemical fluctuations, and triploidy (Taghipoor *et al*. 2016). Although there have been many studies on the effects of triploidy on fish behavior and physiology (for reviews, see Maxime 2008; Fraser *et al*. 2012), far less information is available on the effects of tetraploidy (Babaheydari *et al*. 2016) and none on the effects of tetraploidy on the antioxidant defense systems in rainbow trout, despite its importance as an aquaculture species. The objective of the present study was therefore to investigate how the antioxidant defense system works during early development in tetraploid rainbow trout.

MATERIALS AND METHODS

Experimental fish

Artificial fertilization, first cleavage interval (FCI) analysis, heat shock treatment for ploidy manipulation, and fish rearing were conducted at Abzi Negin Shayan Fereydounshahr Co., Isfahan, Iran. All fish care and handling followed the guidelines of the Iranian Aquaculture Society and the American Institute of Fishery Research Biologists. Broodfish were kept under regular hatchery conditions at 10-11ºC and ambient photoperiod. Gametes were collected from eight females and three neomales 3-year-old rainbow trout, *Oncorhynchus mykiss* broodfish with average weight of 2.3 and 1.8 kg, respectively. The neomale fish were produced by dietary inclusion of 17 α methyltestosterone, 3 mg kg^{-1} for 90 days initiating after exogenous feeding. These fish can only produce milt carrying X chromosome for producing all-female offspring (Cousin-Gerber *et al*. 1989; Arslan *et al*. 2012).

FCI analysis, heat shock treatment, and tetraploid verification

Firstly, the FCI for each rainbow trout female was separately determined based on the methods described by Hershberger & Hostuttler (2005) after collecting about 50 g (~ 600 eggs) and fertilizing them by the mixed milt from 3 neomale fish. The rest of the milt were kept in refrigerator at 4 ºC. The day after FCI determination, the rest of eggs (~ 150 g ~1800 eggs) from each female was stripped and mixed together (n =5) based on the similarity in FCI ~ 5000 degree-min, and the mixed eggs (about 750 g ~ 9000 eggs) were fertilized with the mixed milt. After fertilization, the eggs were divided into two parts, one as control (diploid) group without shock and the other as heat-shocked group served as tetraploid, both group in triplicates. Tetraploidy was induced using late heat shock (28 ± 0.5 ºC for 10 min) starting at 65% FCI, ~325 min post-fertilization at an incubation temperature of ~10 ºC. Heat shock treatment is cheaper and easier to run mainly on small amount of eggs instead of pressure shock which needs expensive apparatus (do Nascimento *et al*. 2020). Batches (n = 3) of control and heat-shocked embryos were incubated in California baskets at 10-11 ºC using spring water. After hatching, they were reared at equal densities (around 5 individuals per 1 L of basket) under the same environmental conditions (10-11 ºC, pH 7.2, and dissolved oxygen not less than 95% air saturation, water hardness, 75 mg L^{-1} $CaCO_3$). At the fry stage

they were fed a trout starter diet (21 Beyza, Fars, Iran) at least 12 times per day, ad libitum. Dead embryos were removed at 10 days post-fertilization and cleared in Davidson's fixative to allow visualization of the neural cord for estimation of initial survival and fertilization success. Further removal of dead individuals at 220, 330, 450, and 750 degree-days post-fertilization (ddpf) was carried out to estimate survival to the eyed, hatching, swim-up, and fry stages, respectively. Live fish were also weighed at hatching, swim-up and fry stages (n=15, from each replicates in nearest 1 mg) and, the final wet weight at fry stage (n = 10, from each replicates in nearest 1 mg) was used to calculate specific growth rate (SGR). Ploidy levels were determined at the eyed and swim-up stages by measuring cellular DNA content (Xavier *et al*. 2017). At the eyed and swim-up stages, 9 samples from control (n = 3 from each replicate) and 30 samples from heat-shocked group (n=10 from each replicate) were analyzed individually. The chorion and yolk sac of the eyed embryos in addition to the abdomen of the swim-up stage were removed before analysis. Tissue lysis was performed by detergent solution (9.53 mM $MgSO_4.7H_2O$, 47.67 mM KCl, 15 mM Tris, 74 mM Sucrose, and 0.8% of Triton X-100), then filtered through 30 µm. An aliquot of 3, 8-diamino-5-6-phenyl-phenanthridinium diiodide (propidium iodide, Cayman chemicals) was used for nuclei staining before analysis by Becton Dickinson flow cytometer (New Jersey, USA). The DNA content of 50,000 to 100,000 individual cells were analyzed per samples by Becton Dickinson flow cytometer.

Antioxidant activity

Samples for antioxidant activity (1-g triplicates at pre-fry stages and 2-g triplicates for fry stage) were randomly collected as fertilized eggs (at 385 min post-fertilization, about 60 min after heat-shock treatment) and at the same times as for survival and growth assessment, then immediately stored in liquid nitrogen. After hatching, the fish were deeply anesthetized with clove powder (150 ppm) and then rinsed with distilled water before freezing.

The samples were homogenized in 7-10 volumes of cold phosphate-buffer saline (PBS, pH 7.4), centrifuged at 3000 g for 10 min at 4 ºC to remove debris, and the supernatant was used for the antioxidant enzyme, vitamin, and malondialdehyde (MDA) assays. Catalase (CAT) activity was analyzed according to the method described by Beers & Sizer (1952) based on decomposing of hydrogen peroxide (H_2O_2). Superoxide dismutase (SOD) activity was assayed using the spectrophotometric method (Ukeda *et al*. 1999) and glutathione peroxidase (GPx) activity by measuring the NADPH oxidation rate following the method described by Bell *et al*. (1985). Non-enzymatic antioxidant molecules, vitamins C, A, and E, were analyzed using the HPLC method as described by Katsanidis & Addis (1999). Malondialdehyde (MDA) levels, known as thiobarbituric acid reactive substances (TBARs), were measured spectrophotometrically at 532 nm according to Buege & Aust (1978).

Statistical data analysis

SPSS 25 for Windows was used to check the normality of the means and variances, and for all subsequent statistical analyses. For survival and growth performance comparison between the control and heat-shocked groups, separate t-tests were used at each developmental stage. Data for antioxidant activity were subjected to One-Way ANOVA followed by Duncan post-test or t-test for means comparisons within and between groups, respectively. Data are shown as means ± SE with $P \leq 0.05$ as the level of significance.

RESULTS

The control group exhibited significantly higher survival rates from fertilization to fry (750 ddpf) than the heat-shocked group (Table 1; $P < 0.05$ at each developmental stage). The fish showed similar growth performances and there were no significant differences in mean individual weight or SGR between two groups (Table 2; $P > 0.05$). Flow cytometry confirmed 80 % tetraploidy for the heated-shocked fish at the eyed and 70% at swim-up stages, so the overall successful tetraploidy induction was ~ 75%. Based on the DNA mass analysis, all control fish were confirmed to be diploid.

Table 1. Mean survival rates of control (100% diploid) and heat-shocked (~75% tetraploid) groups of rainbow trout at early developmental stages.

Survival (%)	Diploid	Heat-shocked
Fertilization	87.31 ± 11.08^a	45.39 ± 8.07^b
Eyed egg	76.67 ± 8.48^a	25.17 ± 4.03^b
Swim-up	69.77 ± 16.27^a	12.37 ± 2.31^b

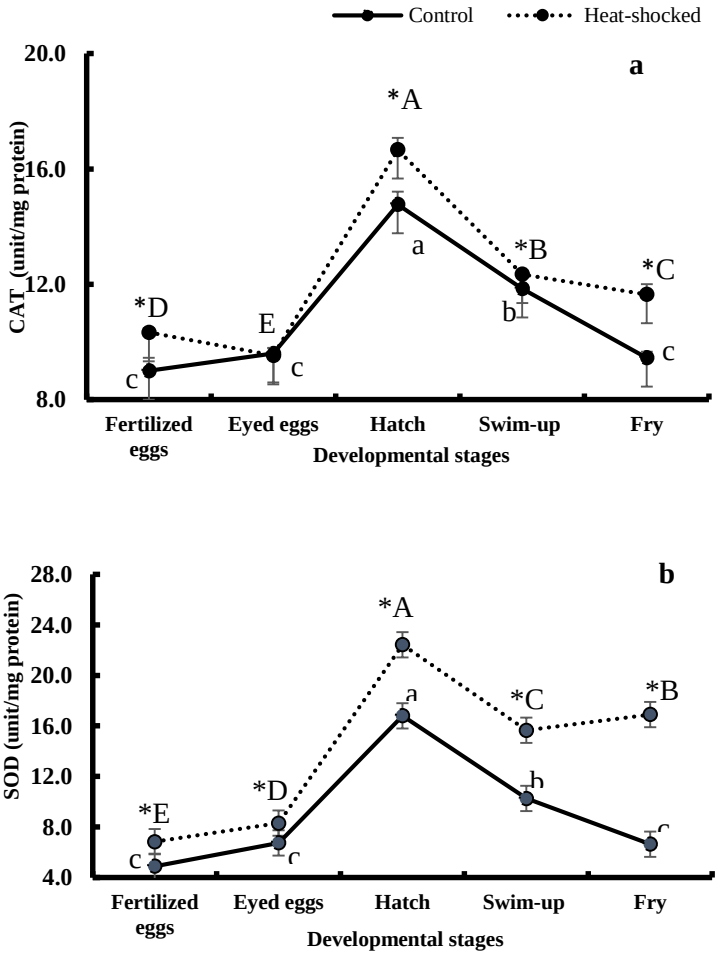
Note: Values are expressed as mean ± SE (n = 3). Mean values with different superscripts within a row are significantly different from each other (*t*-test; $p < 0.05$).

Table 2. Average wet weights (mg) and growth performance of control (100% diploid) and heat-shocked (~75% tetraploid) groups of rainbow trout until 750 degree-days post-fertilization at 10-11°C.

Stage	Diploid	Heat-shocked
Hatch	93 ± 12[a]	98 ± 13[a]
Swim-up	170 ± 25[a]	185 ± 17[a]
Fry	797 ± 80[a]	917 ± 33[a]
Weight gain (mg)	724 ± 56[a]	804 ± 67[a]
SGR (%/day)	4.23 ± 0.31[a]	4.45 ± 0.44[a]

Note: Values are expressed as mean ± SE (n = 45, 15 from each replicate, in triplicate). There were no significant differences between groups at any stage (*t*-test, $p > 0.05$).

In the control group, the CAT activity increased gradually from fertilization to hatching stages, reaching the maximum level of 14.8 ± 0.5 (unit/mg protein), before significantly decreasing to 9.5 ± 0.2 at fry stages (Fig. 1a). The heat-shocked group showed a similar pattern to diploids, although, at all stages, the eyed stage as an exception, showed significantly higher levels of CAT activity in comparison with the diploid compartment (Fig. 1a). At all sampling times, the heat-shocked group showed higher levels of SOD activity than those of the diploid fish (Fig. 1b). In the diploid group, SOD activity was higher only at the hatching and swim-up stages than in the fertilized eggs (Fig. 1b; $P \geq 0.05$). Unlike diploid fish, the SOD activity in heat-shocked group increased during development and displayed significantly higher levels at all sampling times than those of the fertilization stage (Fig. 1b, $P < 0.05$). GPx activity revealed a similar pattern in the diploid and heat-shocked groups (Fig. 1c), where the levels increased gradually and reached the highest levels at swim-up stages, and then began to decrease and reached the basal level (as measured in fertilized eggs) in the heat-shocked group, while in diploids, the GPx activity at fry stage was significantly higher than that measured at the fertilized, eyed, and hatching stages (Fig. 1c; $P < 0.05$).



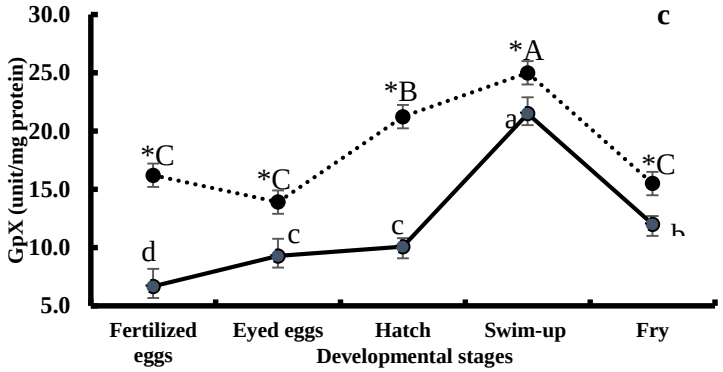
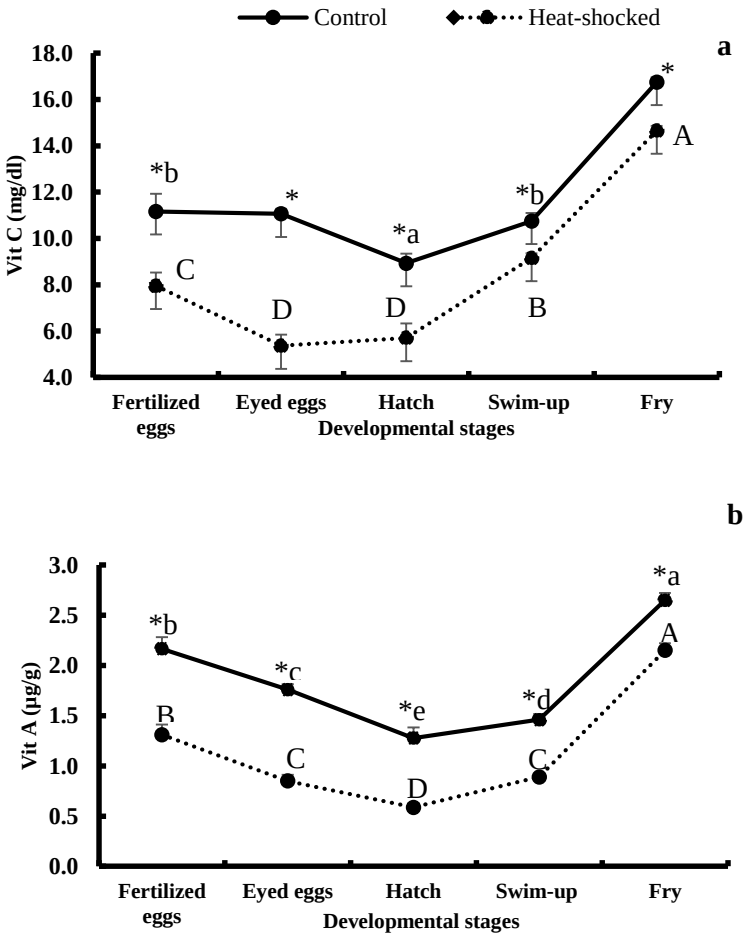


Fig. 1. Activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in the control (100% diploid) and heat-shocked (~75% tetraploid) groups of rainbow trout at early developmental stages. Differences in the same group ($p < 0.05$; Duncan test) or between two groups (diploid and heat-shocked) at the same time (*t*-test) are indicated by the different letters or superscript denotations (*$p < 0.05$), respectively. Values are means (3 replicates) ± SE.

The levels of vitamins C, E, and A were examined during embryogenesis and larval rearing. The levels of all vitamins were significantly reduced from fertilization to the hatching stage in both diploid and heat-shocked groups, then increased significantly after external feeding starting point and reached the highest levels at the fry stage (750 ddpf; Fig 2). The diploid group showed significantly higher levels of all examined vitamins than heat-shocked counterparts at all sampling times (Fig. 2).



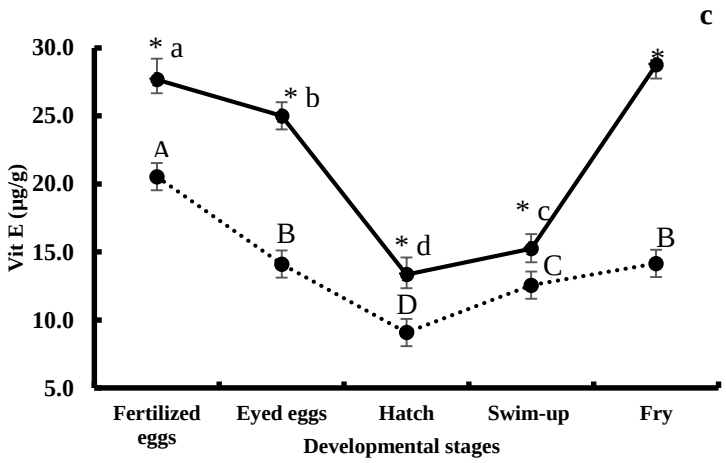


Fig. 2. Levels of vitamin C, A, and E in control (100% diploid) and heat-shocked (~75% tetraploid) groups of rainbow trout at early developmental stages. Differences in the same group ($p < 0.05$; Duncan test) or between two groups (diploid and heat-shocked) at the same time (*t*-test) are indicated by the different letters or superscript denotations (*$p < 0.05$), respectively. Values are means (3 replicates) ± SE.

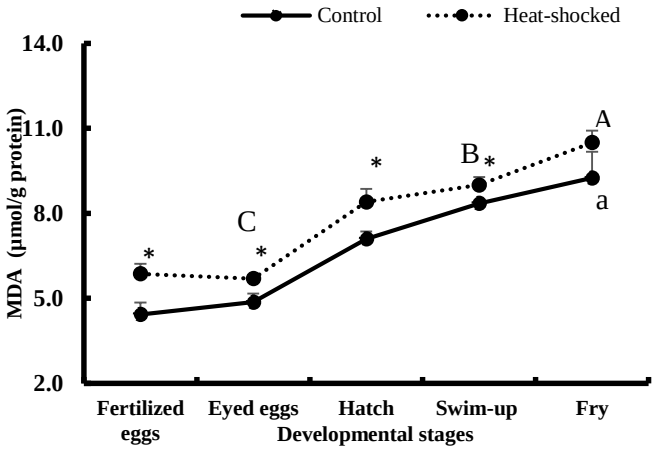


Fig. 3. Levels of malondialdehyde (MDA) in the control (100% diploid) and heat-shocked (~75% tetraploid) groups of rainbow trout at different developmental stages. Differences in the same group ($p < 0.05$; Duncan test) or between two groups (diploid and heat-shocked) at the same time (*t*-test) are indicated by the different letters or superscript denotations (*$p < 0.05$), respectively. Values are means (3 replicates) ± SE.

The lowest MDA levels were observed in fertilized eggs and remained relatively constant until the eyed stage in both diploid and heat-shocked groups ($p > 0.05$; Fig. 3). A steady increase was observed in MDA levels onward in both experimental groups and reached the maximum levels at the fry stage (Fig. 3; $p < 0.05$). The MDA levels were significantly higher in heat-shocked fish at all sampling times (fry stage as an exception) than those of the diploid group (Fig. 3).

DISCUSSION

Application of heat shock to fertilized rainbow trout eggs at 65% of the FCI resulted in a high rate of tetraploidy induction but also a significant reduction in survival rates through all early developmental stages. It is well documented that the viability of tetraploid rainbow trout is lower than that of diploids or triploids (Chourrout *et al*. 1986; Babaheydari *et al*. 2016), while the lower survival and higher deformity rates until the first year of age were also reported in tetraploid brook trout, *Salvelinus fontinalis* (Weber *et al*. 2015). Cell surface alteration to volume, cytological phenomena such as aneuploidy as well as shock-associated detrimental effects are assumed as reasons for low survival rates in tetraploid individuals (Gamal *et al*. 1999; de Alvarenga *et al*. 2020). Significant

alterations in proteome expression related to survival have also been reported in tetraploid rainbow trout (Babaheydari *et al*. 2016). It is worth mentioning that lower survival rates of tetraploid individuals is supported by the differences between tetraploid induction rate at the eyed and swim-up stages after heat shock treatment in our study, showing the higher mortality rates of tetraploids in comparison with the diploid counterparts. Information on the growth performance of tetraploids, in comparison with the triploids, is sparse and not consistent, with reports of lower growth rates in tetraploid rainbow trout (Chourrout *et al*. 1986) and brook trout (Weber *et al*. 2015) compared to diploids, equal growth performance for tetraploids and diploids (Horstgen-Schwark 1993), or better growth for tetraploids (Babaheydari *et al*. 2016). Differences in treatments used to induce tetraploidy, culture conditions, and possible epigenetic modifications associated with the genetic background of the fish (Blanc *et al*. 1993; Farahmand *et al*. 2007) could explain these differences in growth performances. The effectiveness of antioxidant defense systems that protects cells from ROS toxicity during ontogenetic development and early larval rearing, and therefore plays an important role in a fish's early survival and growth performance, varies among developmental stages and is affected by water temperature, oxygen availability, the type of toxin exposure, and dietary factors (Díaz *et al*. 2010; Hostovsky *et al*. 2012; Pérez *et al*. 2020). Taghipoor *et al*. (2016) reported that early heat shock for triploidy induction has significant effects on the levels of antioxidant enzymes in rainbow trout. Our work indicates that the important antioxidant enzymes, i.e., CAT, SOD, and GPx are active during rainbow trout larval development, which is in agreement with the previous studies (Aceto *et al*. 1994; Fontagné *et al*. 2008; Zengin *et al*. 2015). SOD and CAT play key roles in O_2 and H_2O_2 detoxification, respectively, while GPx acts on organic peroxides produced during lipid peroxidation (Peters & Livingstone 1996). We found similar trends for SOD and CAT activities during embryogenesis, with the highest levels at hatching stages, corresponding to the high metabolic and oxygen consumption rates (Sole *et al*. 2004). GPx activity also steadily increased during development and reached maximum levels at the swim-up stage, a bit later than peak SOD and CAT activities. Similar to our results, Fontagné *et al*. (2008), reported that gene expression for antioxidant enzymes increased during rainbow trout embryogenesis, with earlier SOD expression in very early stages followed by GPx expressions between hatching and swim-up, at the yolk sac resorption. Antioxidant enzyme activities tended to be higher in the heat-shocked (i.e., mostly tetraploid) groups compared to diploids at all sampling times, with CAT activity at the eyed stage as the only exception. Higher levels of antioxidant defense enzymes in the heat-shocked embryos and larvae may show stronger signals of the oxidative stress situation, in the chromosomally-manipulated fish. As pointed out earlier, various environmental stressors, such as temperature, can induce oxidative enzyme expression and activity in various fish (Barim & Karatape 2010) and shellfish species (Barim-Oz & Yilmaz 2017). It was found that environmental stressors such as copper sulphate could affect antioxidant defenses of eggs and larvae in goldfish, *Carrasius auratus* (Kong *et al*. 2013). They indicated the concentration- and time-dependent patterns in the fish antioxidnat responses to water-borne copper, at low concentration, while the SOD and CAT activities were inhibited at higher copper concentrations, exhibiting different responses pattern. It is also well-documented that tetraploid fish have larger cells due to the doubling of genome size (Benfey 1999; Piferrer *et al.* 2009), and the increased activity of antioxidant enzymes in tetraploids may be due to this phenomenon (Gao *et al*. 2007) in addition to the additional gene copies of the enzymes. The augmentation of the antioxidant enzymes in tetraploids may reflect their efforts to cope with unfavorable culture conditions. Fish were reared in this experiment at the optimal temperature for diploids, however, it may be due to the different optimal husbandry conditions for tetraploids, as has been demonstrated for triploids (Atkins & Benfey 2008). More recently, Kim *et al*. (2021) found three distinct (SODs) isoform in Siberian sturgeon, *Acipenser baerii* as a primitive chondrostean fish with ~ 250 chromosomes as a functional tetraploid fish. They suggested isoform-dependent roles for the multigene SOD family in antioxidant defenses associated with developmental stages. Collectively, these findings could support differences in enzymatic antioxidant defenses system between diploid and tetraploid counterparts. In our study, all three low molecular weight scavengers (vitamins A, E and C) exhibited similar patterns, however, in contrast to the antioxidant enzyme activities, their levels were reduced from fertilization through hatching and then gradually increased to reach maximum levels at the fry stage. The heat-shocked (mostly tetraploid) groups displayed lower vitamin levels at all sampling times in comparison with the diploids. Vitamins play pivotal roles in different metabolic pathways such as growth, epithelial tissue development, embryonic progression, immune responses, and stress resistance in fish (Palace & Werner 2006; Trichet 2010). Vitamins also provide early antioxidant protection against ROS during ontogenetic development (Cowey *et al*. 1985). It has also been suggested that higher vitamin content in fish eggs is correlated

with healthier larval stages and better survival in fish with longer incubation periods, like salmonids (Lavens *et al*. 1999). In agreement with previous studies, our results revealed increasing vitamin levels after the start of exogenous feeding (Nelis *et al*. 1988), exhibiting that dietary-supplemented vitamins can be absorbed by fish at the earliest stages of feeding (Harsij *et al*. 2020). Diminished vitamin levels in the heat-shocked group could be related to the protective effects of these antioxidant molecules and their role in rebalancing oxidative tension following heat shock. Similar to our results, Taghipoor *et al*. (2016) and Harsij *et al*. (2020) reported a role for vitamins in coping with stress arising from heat shock and sub-lethal ammonia exposure, respectively. At the fry stage, the differences in whole-body content of vitamins between control and heat-shocked fish were most pronounced for vitamin E. This may be related to its superior function as an antioxidant compared to vitamin A (Packer 1991) which has many other metabolic roles besides being an antioxidant (Fontagné *et al*. 2008). Levels of MDA, a lipid peroxidation index, gradually increased during development and were consistently higher in the heat-shocked group, suggesting that the tetraploid fish faced higher oxidative stress. Fish eggs contain polyunsaturated fatty acids (PUFAs) as the main energy source to support embryogenesis, and these are very sensitive to oxidation by ROS (Hemming & Buddigton 1988). Gradual elevation of MDA levels can be explained by elevating ROS production due to the higher oxygen availability after hatching or due to high levels of PUFAs in the yolk or exogenous feed (Dandapat *et al*. 2003). Our results are in good agreement with those of Serigstad (1987) who reported a progressive elevation in O_2 consumption and ROS production during fish larval development. Significant alterations in MDA levels associated with dietary lipid levels, sources, and culture conditions have also been reported (Fontagné *et al*. 2008; Pérez *et al*. 2020). Moreover, a negative correlation between SOD activity and MDA level, as observed after hatching in our study, has been reported earlier (Ahmed *et al*. 2006). The elevated MDA levels in the heat-shocked group may reflect higher levels of PUFA or lipid oxidation. We suspect that the late heat shock treatment for tetraploidy induction initiates lipid peroxidation in rainbow trout. In contrast to our results, Taghipoor *et al*. (2016) reported only higher MDA levels in fertilized eggs subjected to early heat shock (for triploidy induction), while the triploid larval and fry stages exhibited lower MDA levels than diploids. Furthermore, Halliwell & Gutleridge (2007) pointed out that MDA measurement alone could not provide a perfect estimation for lipid peroxidation and it is necessary to check some other parameters such as 4-hydroxynonenal (4-HNE), and acrolein as lipid peroxidation indices. So, further research is warranted to determine the effects of tetraploidy induction on general lipid peroxidation in fish.

CONCLUSION

Overall results indicate that the late heat-shock treatment and/or tetraploidy status in rainbow trout generally causes elevated mortality during early development at least until the fry stage. However, growth performance of the surviving fish was not affected in comparison with the control, diploid group. Our findings also exhibit significant fluctuations of antioxidant enzymes and low molecular weight scavengers (vitamins C, A, and E) during embryonic and larval development. Elevating MDA content during development may reflect the accumulation of lipid peroxidation despite upraising vitamin levels after starting exogenous feeding, which may indicate a more important role of antioxidant enzymes rather than vitamins as antioxidants. Heat shock and resultant tetraploidy induction resulted in the elevated activity of antioxidant enzyme and diminished vitamin levels, suggesting that heat-shock treatment and/or tetraploidy can amplify oxidative stress reactions in rainbow trout at least by the fry stage. These defense mechanisms can counteract ROS production and lipid peroxidation only to some extent, leading to lower survival rates and higher MDA levels in the heat-shocked tetraploid group.

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