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# Determination of triploidy in rainbow trout, *Oncorhynchus mykiss* using erythrocyte measurements.

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ABSTRACT

In this study, effects of thermal shock (26°C) on *Oncorhynchus mykiss* egg at different duration time (5, 10, 15 and 20 min.) to triploid rate was determined. Level of triploid induction for each treatment was evaluated by erythrocyte measurements. For this purpose, the caudal peduncles of 40 fry for each group were cut off and blood smears prepared. The cell and nucleus lengths (major and minor axis) of 30 erythrocytes for each individual were determined with the aid of a microscope camera (with ScopImage 9.0®) and using a micrometer. There was considerable variation in major axis of erythrocytes cell and nucleus both within individual fish and among individuals of a given ploidy. In the major axis length of erythrocyte, cell and nucleus of 10, 15, 20 min thermal shocked groups were statistically significant compared to the control groups ($p<0.05$)

The presented study demonstrated that the increases of triploid yields are correlated to length of erythrocyte major axis. In conclusion, the best triploid yield for *O. mykiss* was at 26°C for 15 min initiated 15 min after fertilization.

## Introduction

Triploidy has been induced in many salmonid species by application of different methods to newly fertilized eggs to prevent extrusion of the second polar body (Galbreath and Samples, 2000). The induction of triploidy in salmonids may attend to avoid the reduced growth and survival losses associated with sexual maturity in normal fish. This problem can be inhibited by producing non-maturing fish with triploidisation, in this regard production of sterile fish is to be useful to the fish industry.

There were different methods that were known to triploidy induction such as, heat or cold shock, hydrostatic pressure and chemical treatment. It is also known that the most popular method is thermal (heat) shock. Different parameters like duration of thermal exposure time, shock temperature and initiation time of shock (Felip et al., 2001), species/strain susceptibility (Moffett and Crozier, 1995) and many physiological factors (Diaz et al., 1993) can affect the triploid yields.

Several methods were reported for the ploidy identification such as, electrophoresis of proteins and examination of morphology (Liu et al., 1978), counting of nucleoli (Howel and Black, 1980), measurement of nuclear and cellular size of erythrocytes (Purdom, 1993). Also direct methods include chromosome counting by karyotype analysis and DNA content determination by flow cytometry (Thorgaard, 1983).

Erythrocyte size from the blood smear is the most suitable method of ploidy identification (Woznicki and Kuzminski, 2002). Therefore, in this study, the triploid yields were determined in the fish fry by measurement of the nuclear and cellular size of erythrocytes on the blood smear.

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## Material and methods

### *Triploidy induction*

The eggs were fertilized in 10°C natural spring water. After several minutes, the fertilized eggs were rinsed in fresh water and waited 10 min for egg swelling. Eggs were thermal-shocked (26°C) at different times (5, 10, 15 and 20 min) for treated groups. Then all groups were immediately transferred to the incubator.

### *Ploidy determination by erythrocytes measurements*

Triploid rates (TR) were determined with four month old fish by measurement of the nuclear and cellular size of erythrocytes. For this purpose, the caudal peduncles of 40 fry for each group were cut off and blood smears prepared.

The major and minor axis of 50 erythrocyte's cell and nuclei were measured for each specimen from dry blood smears with the aid of a microscope camera (with ScopImage 9.0®) and using a micrometer.

### *Statistical analysis*

One-way analysis of variance (ANOVA) was applied to the data. Cell measurements of trial groups compared according to the control group with Dunnett's t-test, 5% significance level. Analysis of variance (ANOVA) was used to compare treatment effects on survival and ploidy among groups, while Student's t-test was used to compare the length between 2n and 3n larvae and the size of erythrocytes in the

## Results

The erythrocytes measurement included diploids mean cell and nucleus lengths of *O. mykiss* (Figure 1). The 10 min (5.89±0.17 and 2.83±0.09), 15 min (6.45±0.13 and 3.17±0.08) and 20 min (6.65±0.12 and 3.26±0.06) groups were significantly ($p<0.05$) different from the control group (5.15±0.06 and 2.46±0.03). The different measurements of erythrocyte of diploid and triploid *O. mykiss* were summarized in Table 1 and showed in Figure 2 and 3.

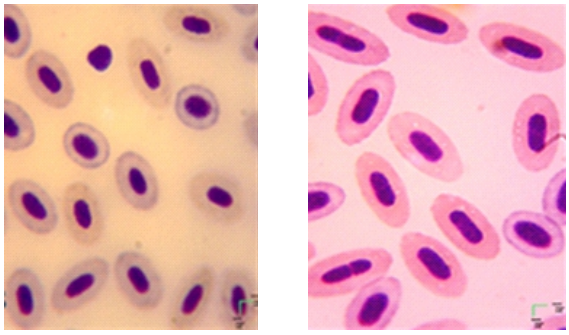

Figure 1. (Left) Diploid (2n) control group erythrocytes; (Right) thermal shock applied triploid (3n) erythrocytes.

Table 1. Effects of thermal shock on erythrocyte size

| | Cell Measurements (µm) | | | |
|---|---|---|---|---|
| **Groups** | **Cell Major Axis** | **Cell Minor Axis** | **Nucleus Major Axis** | **Nucleus Minor Axis** |
| **Control** | 5.15±0.06 | 3.08±0.03 | 2.46±0.03 | 1.34±0.02 |
| **5 min.** | 5.33±0.09 | 3.13±0.03 | 2.54±0.05 | 1.32±0.02 |
| **10 min.** | 5.89±0.17* | 3.22±0.06 | 2.83±0.09* | 1.37±0.02 |
| **15 min.** | 6.45±0.13* | 3.42±0.04* | 3.17±0.08* | 1.37±0.02 |
| **20 min.** | 6.65±0.12* | 3.43±0.03* | 3.26±0.06* | 1.44±0.02* |

Pointed groups with '*' that same fish on same block, it's statistically significant difference obtain according to control group ($p<0.05$).

In this way, there was considerable variation in major axis of erythrocytes cell and nucleus both within individual fish and among individuals of a given ploidy. In the wide axis length of erythrocyte, cell and nucleus of 10, 15, 20 min thermal shocked groups, there were statistically significant differences obtained according to control groups ($p<0.05$). Triploidy yields for 5, 10, 15, and 20 min groups were 8, 29, 59, and 53, respectively (Table 2).

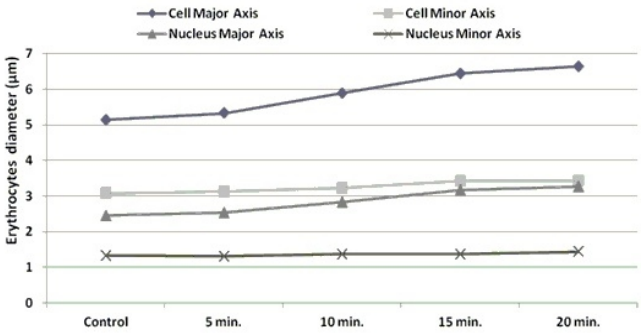


Figure 2. Erythrocyte major and minor axis sizes at different thermal shocked times.

## Discussion

The measurement of the erythrocyte size is an easy and fast method to distinguish the triploid and diploid specimens of different fish species. As described by numerous authors, the erythrocyte nucleus major axis is the most distinct parameter for the ploidy identification in fish species (Benfey et al., 1984; Johnstone and Lincoln, 1986; Boron, 1994, Woznicki and Kuzminski 2002, Dorota et al., 2006). In parallel with this study, Espinosa et al. (2005) concluded that erythrocyte length correctly identified 100% of the fish specimens as diploid or triploid for *O. mykiss*, but they reported that mean nucleus length failed to correctly identify two trouts in the diploid/triploid group.

The use of the automated CASA (Computer-Assisted Analysis) system has the advantage of objectiveness combined with a potential for accurate qualitative evaluation of cells for determined triploidy (Espinosa et al., 2005). According to Woznicki and Kuzminski (2002), triploid and diploid brook trout (*Salvelinus fontinalis*) significantly differ in erythrocyte nuclei length. Therefore, they found that the nuclei measurement is used to determine the ploidy in brook trout. Similarly, triploid saugeyes (female walleye *Stizostedion vitreum* × male sauger *Stizostedion canadense*) had significantly larger erythrocyte cell and nucleus measurements than their diploid counterparts (Garcia-Abiadoa et al., 1999). The cell and nucleus minor axes were found to be poor predictors of ploidy (Benfey et al. 1984). In contrast in present and other studies, Pradeep et al. (2011) have documented a significant difference in cellular and nuclear minor axis which were observed between diploid and triploid erythrocytes in red tilapia (*Oreochromis mossambicus*).

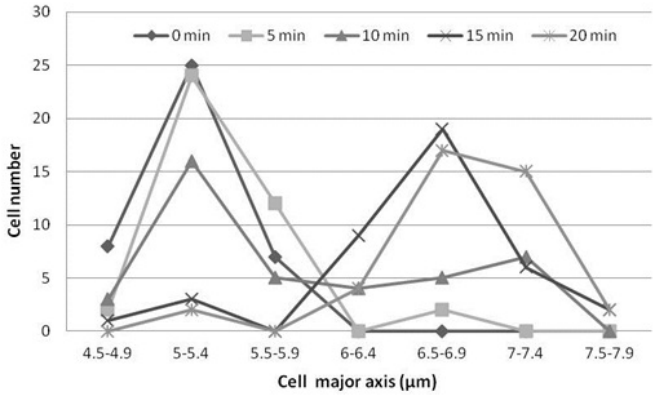


Figure 3. Distribution of the erythrocyte cell major axis length in treatment groups.

Table 2. Effects of thermal shock on triploid yield, survival and triploid rates of O. mykiis

| Groups | Triploid rate (%) | SR (%) | TY (%) |
|---|---|---|---|
| **Control** | 0 | 81.46 | 0 |
| **5 min** | 10 | 75.55 | 8 |
| **10 min** | 40 | 71.25 | 29 |
| **15 min** | 90 | 66.01 | 59 |
| **20 min** | 95 | 55.76 | 53 |

SR: Survival rate, TY: Triploid yield.

The increase in erythrocyte cell and nucleus volume associated with triploidy is mainly a result of an increase in their major axis

(Benfey and Sutterlin, 1984). This can be illustrated by redoubled DNA content. The nucleus and cell are also increased in proportion to it with the polyploidy occurring or genome repeating (Siyang and Suwen, 1987).

## Conclusion

The main purpose of this study was to determine the triploid yield using various erythrocyte indices technique. According to our results, the erythrocyte measurements were able to identify triploid *O. mykiss*. Triploidy is mainly associated with an increase of in the erythrocyte cell and nucleus major axis. But the cell and nucleus minor axes were found to be poor predictors of ploidy. In our experiment, the triploid rate of *O. mykiss* could reach to 95%, while the triploid yield was 53% at 20 min heat shock group. The identification of triploid or diploid rainbow trout can base on these parameters. In conclusion, this study showed that the best triploid yield for *O. mykiss* was at 26°C for 15 min initiated 15 min after fertilization.

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## References

Benfey, T. J., and A. M. Sutterlin. 1984. The Haematology of Triploid Landlocked Atlantic Salmon *Salmo salar* (L.). Journal of Fish Biology, **24**: 333-338.

Benfey, T., Sutterlin, A., and R. Thompson. 1984. Use of Erythrocyte Measurements to Identify Triploid Salmonids. Canadian Journal of Fisheries and Aquatic Sciences, **41**: 980–984.

Boron, A. 1994. Use of Erythrocyte Measurements to Detect Natural Triploids of Spined Loach *Cobitis taenia* (L.). Cytobios **78**: 197–202.

Diaz, N. F., Iyurra, P., Veloso, A., Estay, F., and N. Colihueque. 1993. Physiological Factors Affecting Triploid Production in Rainbow Trout. Aquaculture **114**: 33–40.

Dorota, F. B., Jankunand, M., and P. Woznicki. 2006. Chromosome Number and Erythrocyte Nuclei Length in Triploid Siberian Sturgeon *Acipenser baeri* Brandt. Caryologia **59**: 319-321.

Espinosa, E., Josa, A., Gil, L., and J.I. Marti. 2005. Triploidy in Rainbow Trout Determined by Computer-Assisted Analysis. Journal of Experimental Zoology **303**: 1007–1012.

Garcia-Abiadoa, M. A. R., Dabrowskia, K., Christensena, J. E., Czesnya, S., and P. Bajerb. 1999. Use of Erythrocyte Measurements to Identify Triploid Saugeyes. North American Journal of Aquaculture **61**: 319-325.

Howel, W. H., and D. A. Black. 1980. Controlled Silver Staining of Nucleolus Organizer Regions with a Protective Colloidal Developer: a One-Step Method. Experientia **36**: 1014–1015.

Johnstone, R., and R. F. Lincoln. 1986. Ploidy Estimation Using Erythrocytes from Formalin Fixed Salmonid Fry. Aquaculture **55**: 145-148.

Liu, S., Sezaki, D., Hashimoto, K., Kobayashi, H., and M. Nakamura. 1978. Simplified Techniques for Determination of Polyploidy in Ginbuna, *Carassius auratus* Langsdorfi. Bulletin of the Japanese Society for the Science of Fish. **44**: 601–606.

Moffett, I. J. J., and W. W. Crozier. 1995. An Investigation into the Reproducibility of Triploid Brown Trout, *Salmo trutta* L., Production using Heat Shock. Aquaculture Research, **26**: 67–70.

Pradeep, P. J., Srijaya, T. C., Jose, D., Papini, A., Hassan, A., and A.K. Anil Chatterji. 2011. Identification of Diploid and Triploid Red Tilapia by using Erythrocyte Indices. Caryologia, **64**: 485-492.

Purdom, C. E. 1993. Genetics and Fish Breeding. Chapman & Hall, London.

Siyang, L., and L. Suwen. 1987. On the Erythrocyte Nucleus Size and DNA Contents of *Ctenopharyngodon idellus*, *Megalobrama terminalis* and F, Hybrid. Acta Genetica Sinica, **14**: 142-148.

Thorgaard, G. H. 1983. Chromosome Set Manipulation and Sex Control in Fish. In: Hoar, W.H., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology. Vol. IXB. Academic Press, New York, pp. 405–434.

Woznicki, P., and H. Kuzminski. 2002. Chromosome Number and Erythrocyte Nuclei Length in Triploid Brook Trout (S*alvelinus fontinalis*). Caryologia, 55: 295-298.