Application of DNA flow cytometry for detecting ploidy level of tetraploid rainbow trout (*Oncorhynchus mykiss*)

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Abstract:

Tetraploidization of rainbow trout (*Oncorhynchus mikiss*) was induced by hyperthermia and designed in three treatments (28ºC for 10, 12 and 14 min), 7 h after fertilization. Immature fishes were anaesthetised using standard method in tank (Table 1). Blood samples were collected by caudal venipuncture using 2 mL syringes fitted with 0.5 x 35 mm (25 gauge) needles (Sima, Iran) pre-dosed with heparin (Caspian Tamin, Iran). Blood samples subjected to various conditions, were analysed by FC. Methodology was adapted from established protocols for human blood FC analysis, but modified as required due to the nucleated nature of fish erythrocytes. Blood sample evaluations by FC were performed in surface-deactivated polypropylene tubes to minimize cell-tube adhesion and associated quantitation error. Five millilitre 12 x 75 mm polypropylene Falcon tubes (Maxwell, Italy) were filled with a 4% solution of bovine serum albumin (BSA; Sigma, USA) in PBS and stored overnight at 4°C (Lecommandeur et al. 1994). Prior to use, the tubes were emptied and centrifuged at 2000 rpm for 5 min. For each treatment, we also measured the size of erythrocytes and genome size. Genome size was positively correlated with erythrocyte nucleus size and chromosome number when using PI as the fluorescent dye. This work provides new knowledge on *Oncorhynchus mykiss* genetics/genomics, important for future research in basic cellular/molecular mechanisms and for the development of molecular techniques in this species. However, further investigation is required to obtain a high percent tetraploid Rainbow trout populations.

Keywords: Aquaculture, Flow cytometry, Genome size, Ploidy determination