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COMPARISON OF HEAT AND HEAT-ELECTRO SHOCKS TO INDUCE TRIPLOIDY IN COHO SALMON (*ONCORHYNCHUS KISUTCH*)

by

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ABSTRACT

Teskeredzic, E., E. M. Donaldson, Z. Teskeredzic, E. McLean, and I. I. Solar. 1991. Comparison of heat and heat-electro shocks to induce triploidy in coho salmon (Oncorhynchus kisutch). Can. Tech. Rep. Fish. Aquat. Sci. 1785: 7 p.

This study was conducted to compare the efficacy of a commercially available, thermal and thermal-electro shocking device as method of inducing triploidy in coho salmon. Shocks were applied to eggs for varying duration, 30 minutes post-fertilization. Percent triploid induction was evaluated by flow cytometric analysis. Triploid rates of 100% were produced following combined heat-electro shocks of 10-20 minutes duration. Percent survival of treated eggs was evaluated with reference to control treatments, at the stage of first exogenous feeding. Greatest triploid rate (100%), and highest percent survival (49.2%), were obtained following thermal-electro shock treatment of 10 minutes duration.

Key words: Triploidy, heat shock, electro shock, coho salmon, sterility

RÉSUMÉ

Teskeredzic, E., E. M. Donaldson, Z. Teskeredzic, E. McLean, and I. I. Solar. 1991. Comparison of heat and heat-electro shocks to induce triploidy in coho salmon (Oncorhynchus kisutch). Can. Tech. Rep. Fish. Aquat. Sci. 1785: 7 p.

La présente étude visait à comparer l'efficacité de dispositifs commerciaux de choc thermique et de choc électro-thermique comme méthode d'induction de la triploïdie chez le saumon coho. Des oeufs ont été soumis à des chocs de durée variable, 30 minutes après la fécondation. Le pourcentage d'induction de la triploïdie a été évalué par la technique de cytométrie de flux. Des taux de triploïdie de 100% ont été produits après un traitement combiné de chocs thermiques et de chocs électriques d'une durée de 10 à 20 minutes. Le pourcentage de survie des oeufs traités a été évalué par rapport aux témoins, au stade de la première alimentation exogène. Un taux de triploïdie très élevé (100%) et un pourcentage de survie plus élevé (49,2%) ont été atteints après l'administration de chocs électro-thermiques d'une durée de 10 minutes.

Mots-clés: triploïde, choc thermique, choc électrique, saumon coho, stérilité

INTRODUCTION

Artificial triploidization is a simple form of genetic engineering, which results in the sterilization of teleosts. This technique offers the aquaculture industry a number of practical advantages. For example, triploidization provides a powerful method of inhibiting ovarian development in salmonids. An added benefit of the triploid condition is that energy normally directed toward gonadal growth may be redirected to somatic growth (Utter *et al.*, 1983).

Several methods have been used to sterilize salmonids. Effective but probably uneconomic, are surgical sterilizations (McBride *et al.*, 1963; Brown and Roberts, 1982). Treatments involving post-hatch immersion in, and post-swim up feeding of, appropriate levels of androgens such as 17 -methyltestosterone also induce sterility in Pacific salmon (Goetz *et al.*, 1979; Donaldson and Hunter, 1982). The crossing of tetraploids with diploid individuals, has also been used to generate triploid progeny on an experimental basis (e.g. Chourrout *et al.*, 1987).

The most commonly used techniques of triploid induction employ physical or chemical methods to block extrusion of the second polar body. This has been accomplished following thermal (Chourrout, 1980; Thorgaard *et al.*, 1981; Solar *et al.*, 1984; Johnstone, 1985) and hydrostatic pressure shocks (Benfey and Sutterlin, 1984; Benfey *et al.*, 1988) and by nitrous oxide (Shelton *et al.*, 1986) and cytochalasin B treatments (Refstie, 1981).

To date, however, the effectiveness of electric shock, and combinations of thermal and electric traumas, as an alternative to inducing triploidy in Pacific salmon, has not been examined. Accordingly, we conducted an initial experimental evaluation of the effectiveness of this technology for inducing triploidy in coho salmon (*Oncorhynchus kisutch*). Eggs received treatments for varying durations, 30 minutes post-fertilization.

MATERIALS AND METHODS

Broodstock from Capilano hatchery (North Vancouver, B.C.) were maintained in fresh water (10 ± 0.5 °C) prior to stripping . Ova obtained from two females and sperm collected from two males were pooled and stored in a cooler at 4 °C. Replicate groups of eggs were washed with water + $NaHCO_3$ (1.38 g/l) (Wilcox *et al.*, 1984) and inseminated with sperm in a 500 ml plastic beaker. Eggs were water activated after 10-20 sec, rinsed after 60 sec, and incubated at 10 °C for 30 min prior to shock treatment.

Shocks were applied to duplicate groups on the same day $\leq$ 1 hr post-gamete collection using a commercially produced unit (Clearwater Ltd., Isle of Man, UK). All treatments were completed within 2 hr. The same fertilization and pre-treatment procedures were employed for shocked and control groups. After fertilization, eggs were randomly divided into 18 groups of 120$\pm$ 10. Four replicate groups were immersed simultaneously in a thermoregulated water bath at a temperature of 28 °C, 30 minutes post-fertilization. Another 4 replicate groups received identical thermal exposures, and in addition, an electric shock, following the manufacturer's (Clearwater Ltd., Isle of Man, UK) instructions. Each replicate group was removed after 5, 10, 15 and 20 min treatment. Control groups (120$\pm$10 eggs) were immersed in a water bath maintained at 10 °C for identical periods.

Following treatment, each group was placed into one of 16-compartment plexiglass units with 1 mm plastic mesh bottoms. These units were inserted into Heath Tecna incubation trays supplied with flowing (10 l/min), dechlorinated city water (range 6-10 °C). Incubation was conducted in darkness until 5 days before hatching. 80 ml of 1% malachite solution was added to the top tray every four days as a prophylactic measure. During treatment (30 min) water flow was reduced to 2-3 l/min. Fertilization and survival rates were determined by observation of embryonic development. The number of dead embryos and/or larvae in each group were periodically recorded. All eggs were incubated in subdivided incubation trays until the yolk sac was absorbed (swim up stage). At swim-up , fry (0.28-0.31 g and 36-39 mm) were moved into 50 litre fiberglass tanks subdivided into 4 by a plastic mesh insert.

Blood samples were collected 10 days after the start of first exogenous feeding. Blood was withdrawn, using micropipettes (2 ul), from the severed caudal artery-vein complex of 20 fry (0.34-0.52g, 38-40 mm)/group. Blood was added to a plastic tube containing 0.5 ml of a phosphate buffered saline (PBS)-propidium iodine solution (8.0g NaCl, 0.2g KCl, 1.15g Na_2HPO_4, and 0.2g KH_2PO_4; 50mg [95-98%] propidium iodine/litre, pH 7.4). The samples were stored at 4 °C and, 2 days later, analysed by a fluorescence activated cell sorter (FACS) - Model 440 (Becton & Dickinson SICS) using an 488 argon laser. In this system, the fluorescence of individual erythrocytic nuclei was determined as they passed through a focal point of a microscope objective lens in a modified epi-illumination system.

Triploidy levels were determined by comparing flourescent peaks indicating mean DNA content (based on individual determinations of thousands of cells). A typical sample run from an individual salmon, containing a chick erythrocyte standard, generated a pair of fluorescence pulse-height histograms. The modal value of each pair of fluorescence pulse-histograms was recorded and used to calculate an overall mean mode and 95% confidence intervals for the sample group. The ordinate, Relative DNA Content-Channel, was sufficiently linear such that triploid modal values were 1.5 x higher than diploid values. Triploid yield

was calculated as the product of the triploid rate, and the numbers of individuals surviving to first exogenous feeding, expressed as a percentage of the number of eggs originally present in the group. The chemical characteristics of the water used throughout all stages of experimentation are summarized in Table 1.

RESULTS & DISCUSSION

The present investigations compared the effectiveness of thermal and combined thermal-electric shocks upon triploid induction in coho salmon. All treatments were undertaken using similar aliquots of eggs derived from a common pool, during a 2 hour period. This protocol thereby permits direct comparison of the efficacy of both methods of triploid induction, without the constraint of between-batch "egg fitness" (e.g. ripeness, size, origin etc), which has confounded comparisons of other studies.

The results from the present investigation, which are summarised in Table 2, demonstrate that control eggs, which were immersed for varying times in 10 °C water, exhibited a 93.5% rate of survival to the swim-up stage. This is well within the acceptable range of survivability for commercial and experimental rearing facilities. Although the spontaneous occurence of triploidy in unmanipulated salmonids has been reported (e.g. Guoxiong *et al.*, 1989), FACS-laser determinations revealed zero percent triploid yield for the controls in the present study, when compared against chick erythrocyte standards.

Both heat shock alone, and thermal-electric shock combinations produced triploid animals, but survival and percent triploid induction varied with duration of treatment and form of trauma delivered. Thus, heat shock resulted in decreasing percent survival with increasing treatment duration, which was inversely related to percent triploidization achieved. Highest triploid yield (29.2%) following such treatment was attained with 10 min immersions at 28 °C. Comparison of the results presented with those of similar studies is complicated, since widely varying protocols have been employed, using broodstock of varied origin and perhaps of differing reproductive status. However, the degrees of triploid induction observed in the current investigation, following thermal trauma, are comparable to those achieved by Utter *et al.* (1983) with the same species, and under similar conditions of temperature. Furthermore, Utter *et al.* (1983) also employed identical methods of establishing triploidization; although, in contrast to the present investigation, differing periods of immersion were employed (1 min *versus* 5-20 min).

Simultaneous administration of thermal and electric traumas to coho salmon eggs resulted in a greater percent triploidization than observed following thermal shock alone. 100% triploidization was achieved following 10 and 20 min treatments. Similar to thermal trauma, percent survival decreased with increasing duration of treatment. Triploid yields were always greater following combination procedures than heat shock alone, with highest values being recorded after a 10 min immersion and electric shock (49.2%).

It is readily apparent that the combination procedure evaluated here, provided highest percent triploid induction, survival and hence percent triploid yield in coho salmon. However the data from this preliminary investigation, while indicating the utility of the heat-electro shock system of triploidization, does not provide an indication of optimum treatment regimes, which inevitably, will vary with water quality, and its effects upon conductivity. Since no information regarding the electric current applied to the eggs was available, future studies will examine the effect of varying electric current and different temperature-current regimes upon triploidy induction.

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TABLE 1.

Quality of well-source water used throughout experimental and rearing stages.

Total carbon (mg/l)	Chloride (mg/l)	Conductivity (umho/cm)	Hardness (Ca+Mg) [mg/l]	pH
6	38.4	216	57.3	7.5

TABLE 2.

The effect of thermal shock (28 °C), and thermal and electric shock treatments, over various treatment periods (5-20 min) upon triploid induction in coho salmon. All evaluations were undertaken 30 minutes following artificial fertilization. The presented results are the combined means of replicate groups for each treatment regime. T = % triploid; S = % survival; TY = % triploid yield.

Treatment time (min)	Control*	Thermal Shock	Heat/Electro Shock
5	T = 0 S = 93.5 TY = 0	T = 13 S = 83 TY = 10.8	T = 60 S = 65 TY = 39
10		T = 73 S = 40 TY = 29.2	T = 100 S = 49 TY = 49.2
15		T = 86 S = 32 TY = 27.5	T = 93.3 S = 38 TY = 35.5
20		T = 93.3 S = 14.2 TY = 13.3	T = 100 S = 17.5 TY = 17.5

* Control values represent the pooled means for all treatment times.