

FRED Reports

EXPERIMENTAL PRODUCTION
OF TRIPLOID CHINOOK SALMON
AT DEER MOUNTAIN HATCHERY

by
Carol Denton

Number 74



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ABSTRACT

Triploidy was induced in Unuk River spring chinook salmon, *Oncorhynchus tshawytscha*, at Deer Mountain Hatchery (Ketchikan, Alaska) using heat shock. Immersion in 28°C water for a period of 10 minutes, commencing 5 minutes after fertilization, produced 90%-100% triploids. Subsequent rearing for 53 days showed similar survival but a decreased growth rate, compared to a control group.

KEY WORDS: triploid, sterilization, *Oncorhynchus tshawytscha*

INTRODUCTION

The release of hatchery salmonids that do not mature sexually is currently viewed as a promising enhancement tool. Fish that are physiologically unable to mature sexually do not channel energy into production of gametes and can continue somatic growth. This strategy could be a valuable alternative for maximal utilization of fish in excess of broodstock requirements. There are three methods for producing sterile salmon in a hatchery: (1) administering steroid hormones by immersion and/or feeding, (2) inducing triploidy by hydrostatic pressure, and (3) inducing triploidy by heat shock.

Hormone administration is a lengthy process (up to 5 months) and requires the handling of a substance controlled by the Food and Drug Administration (FDA) (Hunter and Donaldson 1984). Induction of triploidy by hydrostatic pressure requires more elaborate equipment, and fish survival is less than that for the heat-shock treatment (Benfey and Sutterlin 1984a; Lou and Purdom 1984). Triploidy is easily induced by a single heat shock, and success has been reported in several different species: coho, *Oncorhynchus kisutch* (Johnson et al. 1986), fall chinook, *O. tshawytscha*, and pink, *O. gorbuscha*, salmon (Utter et al. 1983); rainbow trout, *Salmo gairdneri* (Solar et al. 1984); and Atlantic salmon, *S. salar* (Johnstone 1985). Heat shock of proper temperature and duration

applied shortly after fertilization prevents extrusion of the second polar body during the second meiotic division. Subsequent meiotic processes are disrupted; the end result is sterility in adults (Johnson 1985). Treatment protocol is species and, possibly, stock specific.

Heat shock was first used on an Alaskan salmonid stock at Snettisham Hatchery, resulting in the successful treatment of coho salmon (Kron et al. 1985).

This study assesses the feasibility of inducing triploidy in spring chinook salmon (Unuk River stock) under existing conditions at Deer Mountain Hatchery. The hatchery is located on Ketchikan Creek, which has a chinook salmon population derived from Unuk River stock.

MATERIALS AND METHODS

Gametes were obtained from five pairs of chinook salmon at Deer Mountain Hatchery on 2 September 1985. Eggs and milt were pooled separately, split into replicates, and stored in plastic bags inside insulated coolers for a period of 3.5 to 5.0 hours. Storage temperatures ranged from 3.5°C to 8.0°C. Immediately prior to fertilization, gametes were removed from the coolers and air-tempered to the required temperature. If necessary, water used in fertilization was also chilled to achieve the required preshock temperature.

Heat shocking was accomplished in the apparatus shown in Figure 1. The base was a styrofoam-insulated Living Stream[®] reservoir (485-liter capacity) containing a heat coil, submersible pump, and thermostat. Two Heath[®] trays rested on top of the reservoir;

[®] Mention of commercial products or trade names does not constitute endorsement by ADF&G, FRED Division.

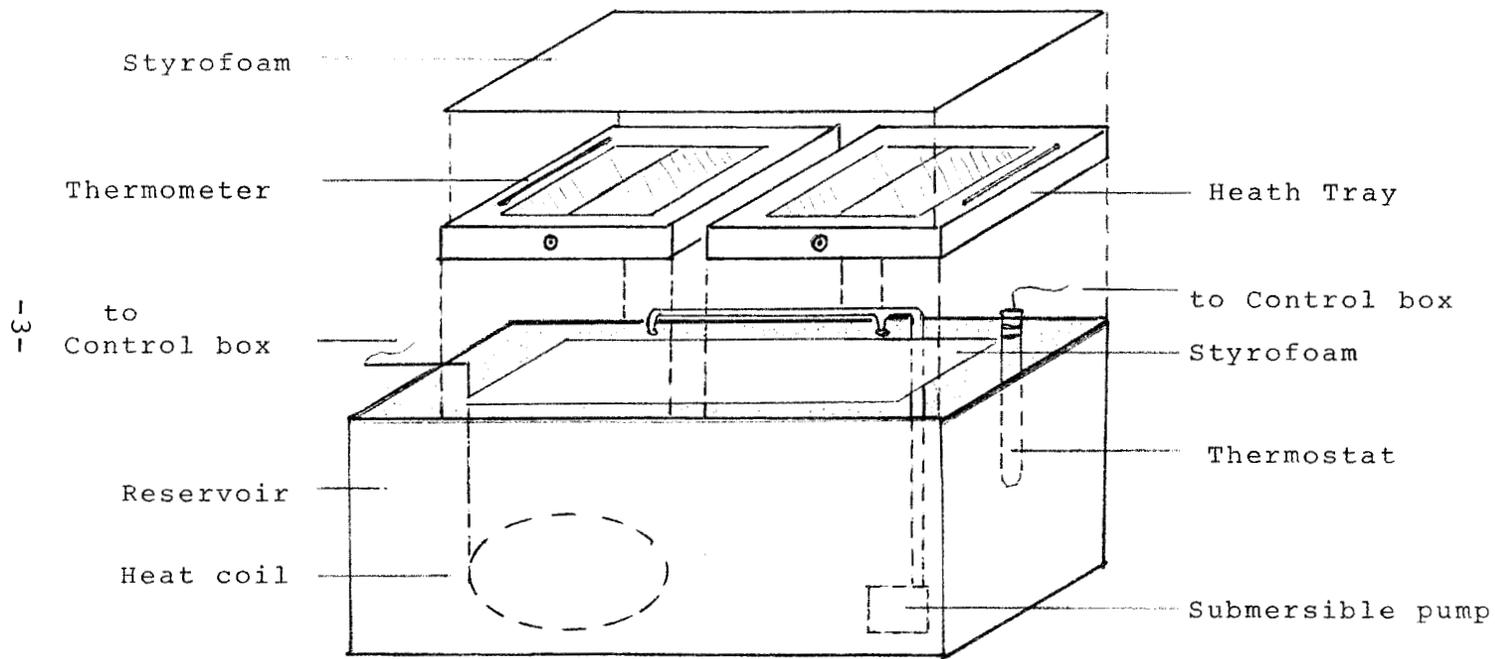


Figure 1. Heat shock apparatus

heated water was pumped from the reservoir through insulated tubing to the trays and then gravity-fed back to the reservoir. Use of the recirculating system prevented low dissolved-oxygen levels during shocking. Water temperature was monitored in the Heath trays and in the reservoir.

Eggs were brought to the Heath trays for shocking in the standard screened inserts. Each screened insert was divided to hold two replicates. After shocking, each entire Heath tray was moved to a stack supplied with ambient water, which when added to the water already in the trays allowed a gradual return to ambient temperature for incubation. Combinations of four variables were tested using six different treatment groups and a control group. The variables were (1) temperature at start of shock, (2) shock temperature, (3) minutes after fertilization, and (4) shock duration. There were two replicates per treatment group; each replicate consisted of approximately 350 ml of eggs.

Samples of emergent fry from each treatment group were sent to Dr. Orly Johnson^{1/} for ploidy determination using flow cytometry (Thorgaard et al. 1982). All fry from the experimental group containing the highest percentage of triploids as well as the control group were reared in modified Edo[®] eyeing containers for a 2-month period. Samples were again assayed for ploidy at the end of rearing.

RESULTS AND DISCUSSION

Table 1 summarizes heat-shock treatment given to the six experimental groups. The physical set-up for heat shocking was

^{1/} National Marine Fisheries Service, Seattle, Washington.

Table 1. Summary of heat shock variables.

Group	Start °C	Minute post-fert.	Shock °C	Shock duration (min)	Δt °C
A	7.0	15	29.5	5	22.5
B	14.0	5	29.5	5	15.5
C	5.7	15	28.0	10	22.3
D	14.0	5	28.0	10	14.0
E	4.0	20	26.5	10	22.5
F	-14.0	10	26.5	10	12.5
Control	14.0	5	14.0	10	0

not totally satisfactory. Stated shocking temperatures are $\pm 0.5^{\circ}\text{C}$, because the temperature in each tray initially fell to 0.5°C below the stated temperature and then increased 1°C before stabilizing at the stated temperature. Temperature fluctuation was due to the large mass of cool objects (eggs and screened-tray insert) relative to the volume of warm water.

Kidney tissue samples sent to the Fisheries Rehabilitation, Enhancement and Development (FRED) Division, Fish Pathology Laboratory in Anchorage were checked for bacterial kidney disease (BKD) using the fluorescent antibody technique. One female and possibly two others were positive for BKD. Eggs remained outside the hatchery building for the entire incubation period, which included two extremely cold periods; extensive ice buildup occurred around the Heath stack.

The eggs were not drop-shocked at the eyed stage because of the possibility they were more impact sensitive than diploid eggs. Hatching occurred at approximately 490 TUs in mid-October. On 16 October it appeared that slightly more of the heat-shocked eggs had hatched than the control eggs (20% vs. 15%). Mortalities were carefully removed from Heath trays on 12 occasions during the 5.5-month incubation period. A count of the two obvious abnormalities, siamese twinning and scoliosis, was kept (Table 2).

Decreasing survival appeared to be positively correlated with decreasing shock temperature; however, these groups were stocked in descending order in the Heath frame, because of the necessity of not stocking a tray of warm water above one that had already returned to ambient temperature. Past incubation records for Heath stacks at Deer Mountain Hatchery indicate no differential survival due to tray position. Extremely cold weather and ice build-up may have caused a progressively decreasing water flow to the lower trays, reducing survival. Percent of abnormal alevins and efficacy of treatment could not be correlated.

Table 2. Relative incubation survival and percent abnormal alevins.

Group	Percent survival	Percent abnormal
A	80.7	3.2
B	79.6	1.0
C	75.4	2.8
D	75.7	2.3
E	72.1	2.8
F	70.3	2.6
Control	100.0	2.4

Random samples of 20 fish for each treatment group were collected after approximately 90% of the fry had buttoned up. Blood from each fish was individually fixed in 2 ml of Alserver's solution (2% glucose, 0.8% trisodium citrate, and 0.4% NaCl) and shipped on ice to Dr. Orlay Johnson for ploidy determination; the results (Table 3) were considered preliminary because of the dilute nature of the samples, and all readings were $\pm 10\%$.

The two groups with the highest percentage of triploids were shocked at 28°C for 10 minutes. The most successful treatment had a Δt of 14, which is less than the Δt values reported elsewhere. Effective Δt s in other studies have ranged from 15 for rainbow trout, *Salmo gairdneri*, (Solar et al. 1984) to 22 for Atlantic salmon, *S. Salar*, (Benfey and Sutterlin 1984b) and coho salmon, *O. kisutch*, (Kron et al. 1985).

Equal numbers of fry from Group D, the most successful treatment group, and the control group were subsequently reared for 53 days. Blood samples from Group D were again fixed in a smaller volume of Alserver's solution and analyzed for ploidy using flow cytometry. All of the samples were triploid. Table 4 gives a comparison of various parameters recorded during rearing.

Although the triploid group showed a decreased growth rate, the increase in condition factor indicates they were feeding reasonably well. The similar survival rates for the triploid and control groups are encouraging. Mortality during the final 2 weeks of rearing was discounted; a sharp increase in mortality in the control group indicated a problem extraneous to the experimental treatment.

Length frequencies in both the experimental and control groups exhibited a bimodal curve, with peaks at 34 and 39 mm at the start of rearing on 20 February (Figure 2). Bimodality was less apparent for the experimental group than for the control group at

Table 3. Percent triploids in samples of emergent fry.

Group	Percent triploids
A	40
B	25
C	78
D	92
E	40
Control	0

Table 4. Size and survival of fry in triploid and control groups during 2 months of rearing.

	Triploid	Control
Weight (g) - Start	0.420	0.407
Weight (g) - End	0.627	0.733
Growth Rate ¹	7.56	11.10
Fork Length (mm) - Start	37.06	36.98
Fork Length (mm) - End	40.47	41.37
K - Start ²	0.825	0.805
K - End	0.946	1.035
Percent Survival 2-20 to 4-5	95.93	94.85

¹ Instantaneous growth rate: $1 \text{ GR} = \frac{1}{t} \text{LN} \left(\frac{\text{WE}}{\text{WB}} \right) \cdot 1000$

Where WE = weight at end, WB = weight at beginning, and t = time in days.

² Condition factor, $K = \frac{W}{L^3} \cdot 10^5$, based on fork length.

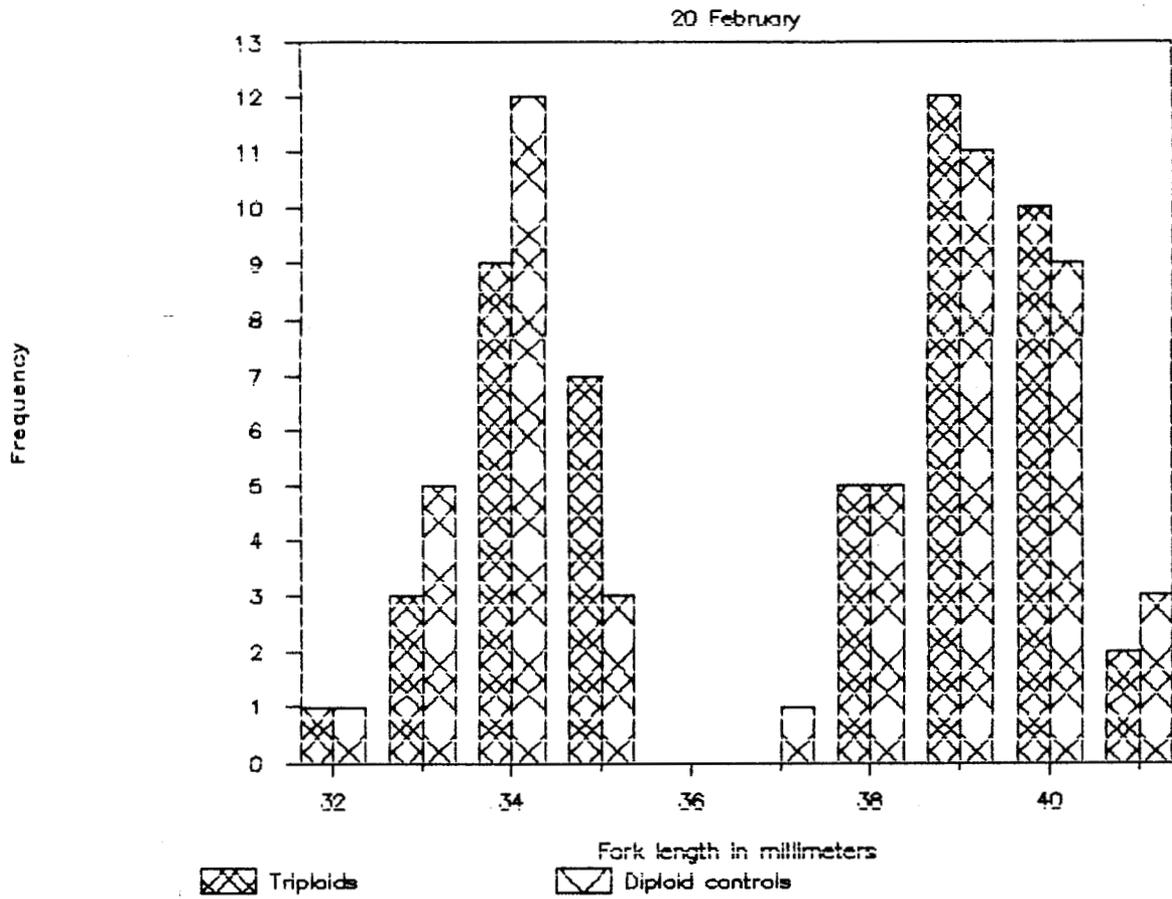


Figure 2. Length frequencies of triploid and diploid chinook salmon prior to rearing.

the end of rearing on 14 April (Figure 3). Biomodality was most likely due to the narrow genetic base for the experiment.

Conclusions

The feasibility of producing triploid spring chinook salmon at Deer Mountain Hatchery has been demonstrated. Exposing eggs to a temperature of 28°C ($\pm 0.5^\circ$) for 10 minutes, commencing 5 minutes after fertilization, produced a 90%-100% triploid group.

Although triploid salmon are functionally sterile, some meiotic activity has been shown to occur (Benfey and Sutterlin 1984b). Also elevated androgen levels at spawning time have been exhibited in rainbow trout (Lincoln and Scott 1984). Triploids are therefore considered to be less completely sterile than fish given appropriate doses of steroid hormone. It is unknown if any behavioral modification will be elicited in triploid Pacific salmon by these small physiological changes. Triploid salmon have been released in the Puget Sound area and will normally return to spawn in 1986 (Fred Utter, pers. comm., NMFS, Seattle). Continuation of the Deer Mountain Hatchery triploid program is contingent upon returns from the Puget Sound releases.

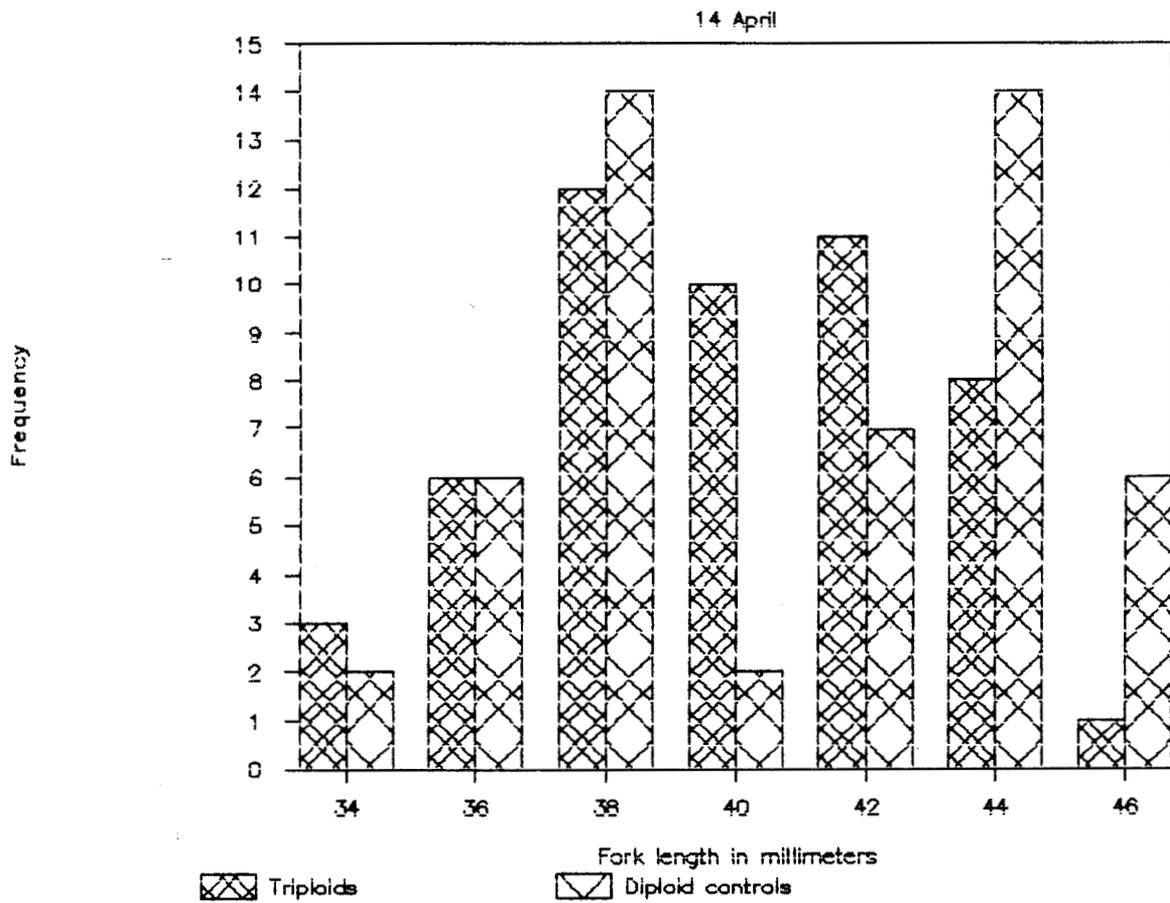


Figure 3. Length frequencies of triploid and diploid chinook salmon after 53 days of rearing.

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