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Use of Estradiol and Methyltestosterone to Change Sex Ratios of Chinook Salmon

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ABSTRACT

Chinook salmon (Oncorhynchus tshawytscha) eggs and fry were treated with steroid hormones. Immersing eggs in 400 µg steroid/l water during incubation significantly ($P < 0.05$) shifted sex ratios from the expected 1:1 ratio: Treatment with methyltestosterone, U.S.P., produced 64% males, and treatment with estradiol, N.F., produced 69% females. Additional estradiol in the diet of fry previously treated with the same steroid did not change sex ratios; however, additional methyltestosterone in the diet of fry previously treated with methyltestosterone significantly increased ($P < 0.01$) the proportion of sterile fry from 0 to 50%. Although survival of eggs and fry was not affected by the treatments, fish treated with estradiol were significantly smaller ($P < 0.05$) at the smolt stage than controls and fish treated with methyltestosterone.

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INTRODUCTION

In Alaska, chinook salmon (Oncorhynchus tshawytscha) migrate to sea in the spring at age 1 or older and remain there for 1-5 yr before returning to their natal river to spawn (Meehan and Siniff 1962; Rowland 1969). Fish that mature after only 1 or 2 yr in the ocean are all males (Kissner 1979). These precocious males, called "jacks," do not contribute to the commercial or sport catch because they are smaller than the legal size limit for these fisheries.

Precocity can be exacerbated during hatchery culture. Chinook salmon have returned to hatcheries in southeastern Alaska the same year as released as smolts (Heard et al. 1979). At the Little Port Walter hatchery, 10-20% of the total returns (catch plus escapement) from the 1976-brood release of Unuk River smolts spent 0-2 yr in the ocean (Hard et al., unpublished manuscript).

Steroid hormones can alter sex differentiation in fishes (for review, see Donaldson and Hunter 1982), and coho salmon (Oncorhynchus kisutch) have been successfully feminized or sterilized with steroid hormones (Goetz et al. 1979; Hunter et al. 1982). Donaldson and Hunter (1982) review potential applications for controlling sex in Pacific salmon (Oncorhynchus spp.). One possible application is reducing the number of precocious males in hatchery-produced chinook salmon by producing all female or sterile fish. Chinook salmon produced by the hatchery would thus contribute more to the fishery.

The purpose of this study was to determine whether concentrations of estradiol, U.S.P., and methyltestosterone, N.F., that effectively altered sex ratios of chinook salmon in British Columbia (George Hunter, Department of Fisheries and Oceans, pers. comm.) would also alter the sex ratios of an Alaskan stock of chinook salmon.

METHODS

In August 1981, the first filial progeny of chinook salmon transplanted to Little Port Walter (LPW) from the Unuk River returned to LPW and were artificially spawned. About 60,000 eyed eggs from these fish were randomly divided into three groups: Estradiol treatment, methyltestosterone treatment, and control. The eggs were incubated in standard Heath[1] trays from fertilization to fry. Dead eggs and alevins were periodically removed from the trays and counted. When the fry were removed from the incubators, they were counted and placed in vertical raceways (Heard and Martin 1979) for culture to smolts. In March 1983, smolts from the three groups were coded-wire tagged and counted. The fish were weighed (wet weight) when first transferred to the raceways and when tagged.

Both alevins and fry were treated with steroid hormones. Alevins were treated twice, just after hatching and 14 days later, with solutions of steroid hormones that were recycled for 2 h through the Heath trays. Stock solutions of methyltestosterone and estradiol were made by dissolving 100 mg of steroid in 100 ml of 100% ethanol. Treatment solutions, 400 µg/l steroid and 0.04% ethanol, were then prepared by adding 40 ml of stock solution to 100 l of water. Mean water temperature during the immersion treatment was 4°C.

Fry in the estradiol group were fed Oregon Moist Pellets (OMP) treated with estradiol from initial feeding for 3 wk. Fry in the methyltestosterone group were fed OMP treated with methyltestosterone from initial feeding for

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[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

10 wk. Solutions of the steroids in 100% ethanol were sprayed on the food so that each kilogram of food contained 5 mg of estradiol or 10 mg methyltestosterone. After the treatments, the fry were fed untreated OMP.

The control group received untreated OMP throughout the experiment. Before feeding was started, a group of 150 fish each was removed from both the methyltestosterone and the estradiol treatment groups and held separately. These fish also received untreated OMP. All groups were fed at the same rate, which varied from 1-4% of the body weight per day, depending on water temperature.

After 180 days of feeding, samples of 100 fish were taken from each group, and the gonads tentively identified under a dissecting microscope as male, female, or hermaphrodite (both an ovary and testis present). Subsamples of 50 male gonads from the two methyltestosterone groups and subsamples of 6 male, 1 hermaphrodite, and 10 female gonads from the control and estradiol groups were fixed in Bouin's solution. The gonad samples were embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin or Gomori's trichrome. The gonads were identified according to the four types described by Goetz et al. (1979): Female (normal ovaries), male (normal testes), sterile (few or no germ cells present), and intersex (oocytes and spermatogenic cells present within the same gonad).

Statistical differences in the sex composition of experimental groups were evaluated using Goodman's test for multiple comparisons among multinomial populations (Goodman 1964). Size of newly emerged fry and smolts were compared using analysis of variance. Scheffé's procedure for all possible comparisons of treatment means was applied when a significant difference in size was indicated (Winer 1971).

RESULTS

Steroid treatment significantly changed sex ratios (Table 1). The control group contained 56% males, but the sex ratio of this group was not significantly different ($P > 0.1$) from the expected 1:1 ratio. Treatment of alevins with estradiol produced significantly more females than the control group ($P < 0.025$) and the expected 50% ($P < 0.05$). Supplemental estradiol in the diet of fish previously treated with estradiol did not change the proportion of females (Table 1). Treatment of alevins with methyltestosterone did not produce significantly more males or steriles than the control group, but the sex ratio of the treatment group was significantly different from the expected 1:1 ratio. Fry fed supplemental methyltestosterone had significantly different sex ratios than the control group ($P < 0.01$) and the group treated only as alevins ($P < 0.01$).

The steroid treatments did not adversely affect survival: overall survival from eyed egg to smolt was 77% for the control, 78% for the estradiol group, and 84% for the methyltestosterone group. Survival from eyed-egg to fry was higher for groups treated with steroids than for the controls, but survival from fry to smolt was similar for all three groups (Table 2).

At both fry and smolt stages, fish treated with testosterone had the highest mean weight, the controls were intermediate, and fish treated with estradiol had the lowest mean weight (Table 2). These differences were not statistically significant for fry size ($P > 0.1$). Smolts from the estradiol group, however, were significantly smaller ($P \leq 0.05$) than smolts in the other groups.

Table 1. Number of chinook salmon according to type of gonad. For groups treated with testosterone, subsamples of gonads were removed from fish without ovaries and prepared for histological determination of sex. Numbers in parentheses are percent of original sample in each category based on both histological and visual determination.

		Estradiol treatment		Methyltestosterone treatment	
	Control	Without food supplement	With food supplement	Without food supplement	With food supplement
			Visual determination		
Number sampled:	100	100	100	100	100
Category:					
Female	44	69	69	28(30)	0(2)
Male	56	30	30	--(64)	--(45)
Hermaphrodite	0	1	1	2(2)	1(1)
Sterile	--	--	--	--(0)	--(50)
Intersex	--	--	--	--(4)	--(2)
			Histological determination		
Number sampled:	10[a]	6[b]	--	47	46
Category:					
Female	5	5	--	1	1
Male	5	5	--	43	21
Hermaphrodite	0	1	--	0	0
Sterile	0	0	--	0	23
Intersex	0	0	--	3	1

[a]Five males, five females (as determined by visual examination) were selected for histological examination.

[b]Five females, one hermaphrodite (as determined by visual examination) were selected for histological examination.

Table 2. Wet weights, numbers, and survival in each treatment group of chinook salmon. Fry were weighed when they were removed from the incubators; smolts were weighed when tagged before release.

Treatment	Average weight (g ± SE)		Eyed-eggs (No.)	Fry (No.)	Smolts (No.)	Percent survival	
	Fry	Smolts				Eyed-egg to fry	Fry to smolt
Control	0.433 ± 0.006	12.14 ± 0.094	17,318	14,062	13,353	81.2	95.0
Estradiol	0.422 ± 0.001	11.42 ± 0.110	21,945	18,249[a]	17,077	83.2	94.3
Methyltestosterone	0.446 ± 0.008	12.28 ± 0.053	20,650	18,934[a]	17,615	91.7	93.8

[a]Percent survival to smolt based on this number less the 150 fry removed before supplemental feeding of steroid hormones.

DISCUSSION

Although the concentrations of steroid hormones used in this study altered the sex ratios of chinook salmon, the efficacy of the treatment was not as high as that reported for coho salmon in Goetz et al. (1979) and chinook salmon in Donaldson and Hunter (1982). Goetz et al. (1979) observed up to 100% feminization of coho salmon treated with estradiol and up to 100% sterilization for fish treated with testosterone.

There are several reasons for differences in results of our study and the results from Goetz et al. (1979). In our study, the frequency of treatment or the concentration of steroid hormones may have been too low to be effective. Feeding supplemental estradiol to the group previously treated with estradiol did not produce more females than only treating alevins with estradiol, and the concentration of estradiol in food and duration of feeding treated OMP probably should have been increased. Feeding supplemental methyltestosterone did produce more sterile fish than only treating alevins with methyltestosterone. The lower temperatures during immersion treatment with steroids (4°C in this study vs. 12°C in Goetz et al. 1979) may also have reduced the effectiveness of the treatment. Furthermore, the two stocks may respond differently to the steroids. As Goetz et al. (1979) noted, "time of natural [sexual] differentiation may vary considerably between species and even between races" of Pacific salmon.

Increasing the frequency of immersion in our study would increase the probability of exposure during a critical period of sexual development; however, increases in concentration of steroid hormones or frequency of treatment could increase detrimental effects. Coho salmon treated with steroid hormones during incubation had increased mortality at 100, 200, and 400 µg estradiol per liter of water and at 400 µg testosterone per liter of

water (Goetz et al. 1979). High concentrations of estradiol have been reported to be lethal for other species of salmonids (Ashby 1957; Funk et al. 1973; Johnstone et al. 1978).

The only negative effect of steroid treatment observed in our study was the smaller size of fry and smolts treated with estradiol. High doses of estrogen have been shown to reduce the growth of pink salmon (Funk et al. 1973), rainbow trout (Salmo gairdneri) (Johnstone et al. 1978), and coho salmon (Goetz et al. 1979). Rainbow trout, however, increased their growth rate after treatment with estrogen was discontinued and eventually grew as large as control fish (Johnstone et al. 1978). In our study, the differences between the smolts treated with estradiol and control smolts may be an artifact of the feeding regime. The group treated with estradiol may not have been fed enough to overcome the smaller fry size associated with estradiol treatment.

Although the efficacy of treatments used in this study was lower than that of other studies, our study demonstrates that this technique can significantly alter the sex ratios of Unuk River chinook salmon. Recoveries of adult treated fish in the fishery or at the weir at Little Port Walter should indicate whether the changes in sex ratio reduced the number of precocious males without affecting marine survival.

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