THE EFFECT OF THREE ESTROGENS ON THE DIRECT FEMINIZATION OF CHINOOK SALMON (*Oncorhynchus tshawytscha*).

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ABSTRACT

Solar, I.I., E.M. Donaldson, and J. Charles. 1994. The effect of three estrogens on the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Can. Tech. Rep. Fish. Aquat. Sci. No. 1955: 8 p.

A single 2-hour immersion was administered to chinook salmon eggs at the time of about 20% hatch in water (7.2 ± 0.75 °C) containing either the naturally occurring estrogen 17ß-estradiol (E_2) or two synthetic estrogens 17α-ethynylestradiol (EE_2) and 14α,15α-methylene estradiol (ME_2). Three doses of each of the estrogens were used: 100, 400 and 1600 µg/l. Immersion in EE_2 produced 89.3 and 100% females at 400 and 1600 µg/l, respectively. Immersion in E_2 and ME_2 at all dose levels did not result in significant modifications of the 1:1 sex ratio. No dose related differences were observed in the mean weight of the fish at six months of age.

RÉSUMÉ

Solar, I.I., E.M. Donaldson, and J. Charles. 1994. The effect of three estrogens on the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Can. Tech. Rep. Fish. Aquat. Sci. No. 1955: 8 p.

Des oeufs de saumon quinnat à un stade d'incubation de 20 % on été soumis à une balnéation de deux heures dans l'eau (7,2 ± 0.75 °C) contenant soit l'oestrogène naturel 17ß-oestradiol (E_2) soit deux oestrogènes synthétiques, 17α-éthynyloestradiol (EE_2) et 14α,15α-méthylène-oestradiol (ME_2). On a employé trois dosages pour chacun des oestrogènes: 100, 400 et 1600 µg/L. L'immersion dans EE_2 a produit 89,3 et 100 % de femelles à 400 et 1600 µg/L respectivement. La balnéation dans E_2 et ME_2, quel que soit le dosage, n'a pas provoqué de modification notable du rapport des sexes de 1:1. On n'a observé aucune différence liée au dosage dans le poids moyen des poissons à l'âge de six mois.

INTRODUCTION

Methods for the control of sex differentiation in Pacific salmonids for aquaculture include, among others, the indirect production of all-female stocks (a two generation process), and the direct feminization of developing embryos by estrogen treatment.

The indirect technique requires the use of androgen treatment during sex differentiation to induce genotypic females to develop as phenotypic males (Hunter *et al*, 1982; Donaldson and Hunter, 1982). This approach has been applied very successfully by the B.C. mariculture industry in the production of monosex female stocks of chinook salmon (*Oncorhynchus tshawytscha*)(Donaldson, 1986; Solar *et al.* 1987; Solar and Donaldson, 1991).

Phenotypic all-female populations also can be produced by direct feminization using natural or synthetic estrogens. The procedure involves immersion of the salmon eggs in a solution of the estrogen at or around the time of hatching. Apart from the timing of the treatment during early development, other important variables are the duration of the immersion treatment, the choice of the steroid and the dose applied. Previous studies on the effect of these variables on the direct feminization of coho and/or chinook salmon, include the works of Goetz el al, 1979, Hunter *et al*, 1986, and Piferrer and Donaldson, 1989a, 1989b, and 1992 (Table 1).

The main reason for the use of the direct feminization technique is to provide all-female populations for production purposes in cases where monosex female sperm for a given stock or the species is not available. A disadvantage however, is that the use for breeding purposes of ova from fish produced by this approach would result in an increased proportion of males in later generations.

In the present study we examined the effect of the naturally occurring estrogen (17ß-estradiol) and two synthetic estrogens (17α-ethynylestradiol and 14α,15α-methylene estradiol) on the feminization of chinook salmon. A single immersion treatment was applied to eggs around the time of hatching using solutions of each of the three estrogens at three dose levels.

MATERIALS AND METHODS

The experiment was conducted January 18, 1991 at the Kokish River Hatchery, IBEC Aquaculture, Pt. McNeil, B.C. The chinook salmon eggs used in this experiment originated from a cross of a Robertson Creek female and a Big Qualicum male.

Groups of chinook salmon eggs (approximately 200 eggs per group at the stage of about 20% hatch) were submitted to a single immersion treatment in a solution of either 17ß-estradiol (E_2) 17α-ethynylestradiol (EE_2) or 14α,15α methylene estradiol (ME_2). The latter hormone had not been tested in fish prior to this study. Three stock solutions were prepared by dissolving 20 mg of each steroid in 20 ml of 95% ethanol. To obtain the desired doses of 100, 400, and 1600 µg/l, 0.5, 2.0 and 8.0 ml of each steroid stock solution was dissolved in 5 liters of hatchery water placed in 9 plastic vessels.

A single 2-hour immersion treatment was carried out by placing the eggs in small perforated boxes inserted in the plastic vessels containing the hormone solutions. Two groups of eggs were also placed in vessels containing hatchery water only, as controls. Air was gently bubbled into the water to provide oxygenation and water/hormone circulation. During the procedure, which was performed outdoors (air temperature was 5.0 °C), the temperature of the water in the covered vessels dropped from 8 °C to 6.5 °C. After the treatment, the eggs still in their respective boxes, were returned to incubation trays in the hatchery supplied with water at 8.0 °C. The eggs completed hatching within the 4 days following the treatment (Jan. 22).

On March 12 the 11 groups of alevins were ponded into separate tanks supplied with hatchery water at 8.5 °C. The fish were sampled at 6 months of age (July 20) for weight (±0.1 g) and length (±0.1 cm). Whole body cross sections were collected from 26-30 fish per group for histological examination of gonadal morphology.

Means and standard deviations were calculated for length, weight and condition factor (K). The condition factor was calculated using the formula $K=W/L^{3.25} \times 1000$ (Vanstone and Market, 1968). Analysis for alteration in sex ratios was performed with the Chi-squared test. Male and intersex fish were combined in one category. Differences (in absolute numbers) from the expected 1:1 ratio were considered significant when $P<0.001$. Linear regression analysis was performed to find the presence of a relationship between the variables mean weight and tank density.

RESULTS AND DISCUSSION

Tables 2 and 3 summarize the data obtained in this experiment including results on gonadal morphology and statistics of size parameters (means and S.D. of weight, length and condition factor K).

The histological analysis of gonadal morphology (table 2) showed that only ethynylestradiol was effective in inducing significant changes in the gonadal phenotype

of the treated fish. 100% females were produced with EE_2 at the dose of 1600 µg/l, while 89.3 % females were observed when a dose of 400 µg/l EE_2 was used.

Higher female to male ratios were observed in all the estrogen treated groups. However, 17ß-estradiol (except for some intersex fish produced with 1600 µg/l) and 14α,15α-methylene estradiol, were not as effective as EE_2 at any of the doses tested.

Observation of growth data (table 3) shows that no differences in size were apparent between the groups as a result of the treatments. A previous study by Piferrer and Donaldson (1992) using estradiol and ethynylestradiol showed that groups that received immersion treatments with either hormone (survival 86.8 and 82.6, respectively) had larger mean weights and length than controls (survival 99%). In the present study one control group (C1), with fewer fish remaining at the time of sampling due to an accidental escape that occurred prior to ponding, contained larger fish than the rest of the groups. Linear regression analysis of the relationship between mean weight and number of fish in the groups at the time of sampling showed an inverse relationship (r= -0.64). Thus, we interpret the weight differences observed in this study as a reflection of tank density during the rearing period.

The proportion of salmon having a female phenotype observed as a result of estradiol immersion was lower in the present study than in the comparable treatment reported by Piferrer and Donaldson (1992) where 72.2% females were observed following a two hour immersion in 400 µg/l E_2. The reason for this may be that the treatments and further incubation were done using water at 10 °C, while in this experiment the temperature of the water was 8 degrees Celcius during incubation and decreased from 8 degrees to 6.5 degrees during the 2 hours of the immersion treatment.

Another possible cause of the variation may be that the immersions applied by Piferrer and Donaldson (1992) took place 3 days after median hatch, thus all the alevins were exposed to the effect of the hormone without the impairment of the protective chorion, while our experiment took place earlier, at a time when only 20% of the eggs had hatched. Concurrently, because of the combined effect of the lower temperature and earlier timing, a dose 4 times as high was required to obtain the same result achieved by Piferrer and Donaldson (1992) using a 2-hr long immersion in ethynylestradiol.

In conclusion, this study confirms the higher estrogenic potency of ethynylestradiol, relative to the natural estrogen 17ß-estradiol, and further reinforces the notion that timing and environmental conditions are very important for the success of treatments for the control of gender in salmonids.

The estrogens used in this study are not yet approved for use in fish destined for human consumption. The Bureau of Veterinary Drugs, Health and Welfare Canada (H&W), is the federal agency that regulates the use of these bio-chemicals. H&W regulations allow for the use of these compounds by prescription under an "Emergency Drug Release" issued by a veterinarian with the approval from Health and Welfare. It is expected that the estrogens may eventually be cleared and registered for use in aquacultured fish. Even when approved, estrogens should only be used by qualified personnel and care should be exercised to avoid skin contact, inhalation and ingestion, including the use of rubber gloves, mask and protective clothing.

REFERENCES

Ashby, K.R., 1957. The effect of steroid hormones on the brown trout (*Salmo trutta*, L.) during the period of gonadal differentiation. J Embryol. Exp. Morph., 5: 225-249.

Donaldson E.M. and Hunter G.A., 1982. Sex control in fish with particular reference to salmonids. Can. J. Fish. Aquat. Sci. 39: 99-110.

Goetz, F.W., Donaldson, E.M., Hunter G.A. and Dye H.M., 1979. Effect of estradiol-17β and 17α-methyltestosterone on gonadal differentiation in the coho salmon (*Oncorhynchus kisutch*). Aquaculture 17: 267-278.

Hunter, G.A., Donaldson E.M., Goetz, F.W. and Edgell, P.R., 1982. Production of all-female and sterile groups of coho salmon (*Oncorhynchus kisutch*) and experimental evidence for male heterogamety. Trans. Am. Fish. Soc., 111: 367-372.

Hunter, G.A., Solar, I.I., Baker,I. and Donaldson, E.M., 1986. Feminization of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) by immersion of alevins in a solution of estradiol-17ß. Aquaculture 53: 295-302

Nakamura, M., 1981. Feminization of masu salmon, *Oncorhynchus masu*, by administration of estradiol-17ß. Bull. Japan. Soc. Sci. Fisheries 47: 1323-1327.

Piferrer, F. and Donaldson, E.M., 1989a. Hormonal sex control in Pacific salmon: The importance of treatment timing. In: M. Carrillo, S. Zanuy and S. Planas, Compilers, XIth Int. Symp. Comp. Endocrinol. Proc. Satellite Symp.

Applications of Comparative Endocrinology to Fish Culture. Almunecar, Spain, May 22-23, 1989. p. 81-88.

Piferrer, F. and Donaldson, E.M., 1989b. Gonadal differentiation in Coho salmon *Oncorhynchus kisutch*, after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquaculture, 77: 251-262.

Piferrer, F. and Donaldson E.M., 1992 . The comparative effectiveness of the natural and a synthetic estrogen for the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 106: 183-193.

Simpson T.H., 1975/76. Endocrine aspects of salmonid culture. Proc.: R. Soc. Edinburgh, Ser.B 75: 241-252.

Solar, I.I., Baker I.J. and Donaldson, E.M., 1987. Experimental use of "female sperm" in the production of monosex female stocks of chinook salmon (*Oncorhynchus tshawytscha*) at commercial fish farms. Can. Tech. Rep. Fish. Aquat. Sci. No. 1552. 14 p.

Solar, I.I., and Donaldson E.M., 1991. A comparison of the economic aspects of monosex chinook salmon production versus mixed sex stocks for aquaculture. Proceedings, Aquaculture Canada '91, Annual Meeting Aquaculture Assoc. of Canada. , St. Andrews, N.B. June 10-16, 1991. 91-3:28 - 30.

Vanstone, W.E. and Markert, J.R. 1968. Some morphological and biochemical changes in coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation. J.Fish. Res. Board Can. 25: 2403-2418.

TABLE 1. Use of Estrogens in studies of direct feminization in salmonid species.

Species	Estrogen	Dose	No. of Immersions/ duration	Timing	Effect	Reference
Salmo salar	Estradiol	250 µg/l (+feeding)	2/ 2 hr	eyed eggs and alevins (+ feeding for 120 days)	100 % females	Simpson (1975/76)
Salmo trutta	Estradiol	50 or 300 µg/l	1/ 70 or 111 days	alevins starting 170 days from fertilization	high dose: 7 females, 3 males	Ashby (1957)
Oncorhynchus mykiss	Estradiol	250 µg/l (+ feeding)	2/ 2hr	eyed eggs and alevins (+ feeding for 30 and 56 days)	100 % females	Simpson (1975/76)
O. kisutch	Estradiol	25-400 µg/l	2-6/ 2hr 2-7/ 2hr	eyed eggs alevins	60-95% females	Goetz *et al* (1979)
	Estradiol	200 µg/l	2/ 2 hr	eyed eggs and alevins	>99% females	Donaldson and Hunter (1982)
	Estradiol	200-1600 µg/l	2/ 2hr	438 & 508 ATU(1)	87-97% females	Hunter *el al* (1986)
	Estradiol	400 µg/l	1/ 2hr	295-938 ATU	up to 84% females	Piferrer and Donaldson (1989b)
O.tschawystsha	Estradiol	200-1600 µg/l	2/ 2 hr	533 & 603 ATU	66-91% females	Hunter *et al* (1986)
	Estradiol	400 µg/l	1/ 1-8 hr	548 ATU	100% females (8 hr)	Piferrer and Donaldson (1992)
	Ethynylestradiol	400 µg/l	1/ 1-8 hr	548 ATU	100% females (2 hr)	Piferrer and Donaldson (1992)
O. masu	Estradiol	0.25-200 µg/l	1/ 18 days	alevins starting 5 days post-hatch	100% females 0.5-5 µg/l high mort. 10-200 µg/l	Nakamura (1981)

(1) ATU = Accumulated thermal units: degree Celcius days

TABLE 2. Effect of a single 2-hr estrogen immersion treatment (applied at about 20% hatch) on the sex differentiation (percent gonadal morphology) of chinook salmon alevins.

TREATMENT	TREATMENT DOSE	SAMPLE SIZE	GONADAL MORPHOLOGY					
			MALE	%	FEMALE	%	INTERSEX	%
ESTRADIOL	100	27	11	41.7	16	59.3	0	0.0
	400	27	10	37.0	17	63.0[b]	0	0.0
	1600	26	7	26.9	16	61.5[b]	3	0.0
ETHYNYL ESTRADIOL	100	27	6	22.2	14	51.8	7	25.9
	400	28	3	10.7	25	89.3[a]	0	0.0
	1600	30	0	0.0	30	100.0[a]	0	0.0
METHYLENE ESTRADIOL	100	26	11	42.3	15	57.7	0	0.0
	400	28	14	50.0	14	50.0	0	0.0
	1600	27	10	37.0	17	63.0[b]	0	0.0
CONTROL (C1)	0	27	11	40.7	16	59.3	0	0.0
(C2)	0	27	13	48.2	14	51.8	0	0.0

Age at sampling: 6 months; significance of difference from 1:1 male:female ratio: a) $P<0.001$, b)$P<0.25$ (Chi-squared test).

TABLE 3. Weight, length and condition factor (K) of chinook salmon treated with a single 2-hr estrogen immersion.

TREATMENT	TREATMENT DOSE µ/l	SAMPLE SIZE	WEIGHT g	±SD	LENGTH cm	±SD	CONDITION FACTOR	±SD
ESTRADIOL	100	25	7.80	1.53	8.97	0.52	6.17	0.26
	400	25	7.81	1.12	9.02	0.37	6.10	0.34
	1600	25	8.29	1.32	9.06	0.47	6.40	0.49
ETHYNYL	100	25	7.64	1.22	8.94	0.41	6.14	0.47
ESTRADIOL	400	25	7.56	1.25	8.88	0.42	6.20	0.24
	1600	25	7.71	0.80	8.97	0.31	6.16	0.39
METHYLENE	100	25	8.25	1.25	9.16	0.41	6.12	0.26
ESTRADIOL	400	25	7.23	1.20	8.83	0.48	6.04	0.33
	1600	25	7.57	1.13	8.97	0.39	6.02	0.24
CONTROL (C1)	0	25	9.40	1.48	9.49	0.61	6.23	0.43
(C2)	0	25	7.84	1.07	8.96	0.33	6.26	0.38

Age at sampling:6 months; Condition factor: $K = \text{weight} \times 1000 / \text{length}^{3.25}$