Aquaculture, 43 (1984) 75—81
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

EFFECTS OF ESTRADIOL AND DIETHYLSTILBESTEROL ON SEX REVERSAL AND MORTALITY IN ATLANTIC SALMON (*SALMO SALAR*)

STACIA A. SOWER[1]*, WALTON W. DICKHOFF[2], THOMAS A. FLAGG[2], JAMES L. MIGHELL[2] and CONRAD V.W. MAHNKEN[2]

[1] *Department of Zoology, University of Washington, Seattle, WA 98195 (U.S.A.)*
[2] *National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Seattle, WA (U.S.A.)*

*Present address: Department of Zoology, University of New Hampshire, Durham, NH 03824, U.S.A.

ABSTRACT

Sower, S.A., Dickhoff, W.W., Flagg, T.A., Mighell, J.L. and Mahnken, C.V.W., 1984. Effects of estradiol and diethylstilbesterol on sex reversal and mortality in Atlantic salmon (*Salmo salar*). *Aquaculture*, 43: 75—81.

Atlantic salmon alevins were fed diets containing different doses of estradiol (20 or 2 mg/kg diet) or diethylstilbesterol (10, 1, or 0.1 mg/kg diet) for 60 days following yolk-sac absorption in an attempt to produce a monosex female population. Diethylstilbesterol or estradiol at the doses tested did not induce a greater percentage of females by April 1982, when the fish were 14 months old. However, at this time, in all estrogen treated groups, 5—17% of the fish had both ovarian and testicular tissue (intersex fish).

Treatment of salmon with the high doses (20 mg/kg diet) of estradiol or diethylstilbesterol resulted in high mortalities, indicating that high doses of estradiol are toxic to the fish. We conclude that steroid treatment during the fish's developmental periods may not be feasible because of undesirable long-term effects.

In January 1983, when the fish were 12 months old, 50% of the male controls were sexually precocious. By April 1983, 72% of the male controls had testes that were undergoing regression. We suggest that the sexual precocity observed in males in this study may be due to the 12L:12D photoperiod in which they were reared.

INTRODUCTION

Techniques for the control of sex differentiation have been used successfully in several fish species. In salmonids the ability to produce totally female populations could potentially increase the number of eggs available for salmon broodstock programs and eliminate the incidence of precocious sexual maturation of genotypic males. Sex differentiation commences in most species of salmon shortly after hatching or after the initiation of feeding (Donaldson and Hunter, 1982; Yamazaki, 1983). Estrogens are used to induce sex reversal of males to females during this

0044-8486/84/$03.00

time. The treatment with estrogen is usually by feeding the steroid in the diet and/or immersion of the eggs or alevins in water containing the steroid. The dosage, timing, and duration of treatment may depend on species and environmental conditions of temperature and photoperiod.

Treatment of fish through inclusion in the diet and/or immersion has been effective in sex reversal of genetic males to phenotypic females in *Salmo gairdneri* (Jalabert et al., 1975; Johnstone et al., 1978); *Oncorhynchus kisutch* (Goetz et al., 1979); *Salmo salar* (Johnstone et al., 1978); *Oncorhynchus masou* (Nakamura, 1981); and *Oncorhynchus tshawytscha* (Donaldson and Hunter, 1982). However, further studies are required to fully evaluate the effectiveness of various treatment regimes using different genetic stocks reared under differing environmental conditions. Johnstone et al. (1978) reported that the results of sex-reversal studies in rainbow trout yielded different results depending on whether the studies were conducted in the hatchery or in the laboratory. Trout in the hatchery were completely converted to females when they were treated with 20 mg/kg diet of estradiol for 30 days. The same treatment of trout in the laboratory yielded a population of 64% females. These authors attributed this difference in the effectiveness of steroid treatment to differences in photoperiod and fish density.

Our objectives in this study were to evaluate various doses of estradiol and diethylstilbesterol in producing monosex female populations in Atlantic salmon, to determine if these changes were irreversible, and to determine the effects of these steroids on the fish's survival.

MATERIALS AND METHODS

Atlantic salmon of the Gaspe's 1980 Broodstock were held at the Northwest and Alaska Fisheries Center (National Marine Fisheries Service) during the duration of the experiment. Atlantic salmon were transferred following the absorption of yolk sac to twelve 40-l cylindrical tanks in March 1981 and started on their treated diets. Each tank contained 200 salmon randomly selected from a larger population. The fish were maintained in dechlorinated municipal water. Temperature ranged from 7 to 15°C; photoperiod consisted of 12 h light and 12 h dark. The fish were fed Oregon Moist Pellets (OMP) four or five times daily to satiation for the duration of the test period. The treatments were duplicated and consisted of (1) control; (2) 20 mg/kg diet of estradiol; (3) 2 mg/kg of estradiol; (4) 10 mg/kg diet of diethylstilbesterol (DES); (5) 1 mg/kg diet of diethylstilbesterol; or (6) 0.1 mg/kg diet of diethylstilbesterol. The fish were fed treated diets for 60 days, after which they were fed untreated control diets. The treated diets were prepared by adding the test compounds to the oil fraction of the diet before mixing with the other ingredients. Lengths and weights of 10—20 individual fish from each tank were recorded in September 1981, January 1982, and May 1982.

In September 1981, January 1982, and May 1982 gonads from 10 or 20 fish from each tank were preserved in Bouin's solution, embedded in paraffin, sectioned at 10 μm, and stained with hematoxylin and eosin for histological evaluation.

Data for weight were analyzed by a Student—Newman—Keuls test after preliminary analysis of variance. Chi-square analysis was used to analyze sex ratio or sexual precocity data of the treatment groups (observed) and the control group (expected).

RESULTS

There were no measurable differences of weight, sex ratio, or mortality data between the replicates of the treatment groups; therefore, the data for each treatment group represent the duplicated treatments. Neither estradiol nor DES at the doses used was effective in producing all-female populations of salmon (Table I). The initial histological evidence obtained in September 1981 indicated that estradiol at 20 mg/kg diet and DES at 0.1 or 1.0 mg/kg produced 70—95% females. In January 1982 DES (0.1) and estradiol (20) treated fish contained 65% and 85% females, respectively (Table I). At this time, DES (10) groups contained 40% females and 50% intersex (consisting of both testicular and ovarian tissue). Estradiol (20) treated fish also contained 5% intersex. However, by April 1982 all estrogen treated groups were comprised of 5—17% intersex fish, and the male:female ratio was about equal in all groups except for the estradiol (20) treated fish which contained 68% females (Table I).

Mortalities were higher in groups treated with the high doses (20 mg/kg)

TABLE I

Percent of fish containing ovaries, testes and ovo-testes (intersex) with dietary diethylstilbesterol (DES) or estradiol (E_2) compared to controls

Treatment (mg/kg diet)	January 1982[a] (%)			April 1982[b] (%)		
	Sex phenotype			Sex phenotype		
	Female	Male	Intersex	Female	Male	Intersex
Control	40	60	0	52	48	0
DES (10)	40	10[c]	50[c]	43	40	17[c]
DES (1)	25[c]	75[c]	0	48	40	12[c]
DES (0.1)	65[c]	35[c]	0	45	50	5
E_2 (20)	85[c]	10[c]	5	68[c]	15[c]	17[c]
E_2 (2)	50	50	0	35	48	17[c]

[a]Data based on 20 fish/treatment.
[b]Data based on 40 fish/treatment.
[c]Significantly different from controls at $P < 0.05$.

of DES and estradiol by September 1981 (Table II). Interestingly, there were no apparent differences in survival among the hormone treated fish during the 60-day treatment period. Most of the mortalities occurred between 60—120 days. The fish in the DES (10) and estradiol (20) groups weighed significantly more than control fish by April 1982. We attribute this difference to the lower densities that resulted from higher mortalities in these groups rather than to any growth-promoting influence of the steroids.

In January, 50% of the male controls (12 months old) were sexually precocious, as judged by the emission of milt in response to gentle pressure to the abdomen. Histological evidence indicated that the testes lobules were filled completely with spermatozoa (Table III). However, by April 1982, 72% of the male control had testes that had started to undergo re-

TABLE II

Percent survival and body weight of Atlantic salmon treated with dietary diethylstilbesterol (DES) or estradiol (E_2) compared to controls

Treatment (mg/kg diet)	% Survival		Weight[a] — April 1982
	Sept. 1981	May 1982	$X \pm SE$
Control	65	55	114 ± 3
DES (10)	26	14	129 ± 4[b]
DES (1)	67	58	107 ± 3
DES (0.1)	69	60	112 ± 3
E_2 (20)	40	30	122 ± 4[b]
E_2 (2)	69	63	111 ± 3

[a]Data based on sample of 40 fish/treatment.
[b]Significantly different from controls at $P < 0.05$.

TABLE III

Incidence of advanced testicular development in control and steroid treated Atlantic salmon

Treatment (mg/kg diet)	No. of males			
	January 1982		April 1982	
	Normal	Precocious	Normal	Regressed
Control	8	4	11	8
DES (10)	2	0	16	0
DES (1)	15	0	8	0
DES (0.1)	7	0	19	1
E_2 (20)	1	1	6	0
E_2 (2)	9	1	17	2

gression, containing only a few spermatozoa and spermatocytes. Of the steroid treated groups, only estradiol at 20 and 2 mg/kg and DES at 0.1 mg/kg had a few males that had testes in an advanced state of development.

DISCUSSION

The present study is at variance with the only other reported study in Atlantic salmon where dietary treatment alone of estradiol was effective in inducing 100% females (Johnstone et al., 1978). Goetz et al. (1979) found that complete sex reversal to females in coho salmon required immersion of the eggs and alevins in addition to dietary treatment; dietary treatment alone was partially effective. Immersion of Atlantic salmon eggs and alevins and dietary treatment of estradiol at 20 mg/kg diet for 120 days were successful in producing an all-female population (21 fish) after 16 months (Simpson et al., 1976). The combined treatment may be necessary for Atlantic salmon. Our studies clearly show that DES or estradiol at the doses treated did not induce a greater percentage of females; in fact, the ratio of male to female was about equal discounting the intersex fish which appeared in all estrogen treated fish. However, earlier in the experiment, estradiol (20 mg/kg) and DES (0.1 mg/kg) contained higher percentages of females; but after 12 months, this was not the case. The increase in intersex fish and the lack of any group containing a higher percentage of females possibly indicate that the gonads may revert back to that expected based on the sex genotype.

Treatment of salmon with the high dose (20 mg/kg) of estradiol or diethylstilbesterol resulted in high mortalities. Immersion treatment with estradiol of *Salmo trutta* or *Oncorhynchus kisutch* resulted in high mortality (Ashby, 1957; Goetz et al., 1979). Dietary estradiol at 20 mg/kg has also been shown to induce higher mortalities than controls in brook trout (*Salvelinus fontinalis*) (Johnstone et al., 1979). DES (5 mg/kg) or estradiol (5 mg/kg) treated fish had higher mortalities in *Salmo gairdneri* (Sower et al., 1983). The mechanism of estrogen-induced mortality in fish is not known. It is known in other vertebrates that high doses of estrogens (estradiol or DES) inhibit liver and kidney function and that estrogens are involved in the development of animal neoplasia (Grollman, 1964; Serra et al., 1983). Evidence that DES is a cancer-causing agent was shown by the incidence of vaginal and cervical cancer in the progeny of human mothers who had received DES during pregnancy (Herbst et al., 1971; Barber and Sommers, 1974). The several previous studies mentioned, where fish administered high doses of estrogen had high mortalities, would seem to indicate that, like in other vertebrates, estrogens can be toxic. Studies of the actions of estradiol on liver and kidney function and neoplasia have not been reported for fish. If fish respond negatively in their reactions to high doses of estrogens or other sex steroids similar to the responses of other vertebrates, the application of these steroids for sex reversal and sterility may be limited.

Low doses of estrogens may also have detrimental effects on fish. Estrogens have been implicated in evoking migratory behavior (Baggerman, 1960). A recent study suggests the involvement of estradiol in smoltification (Sower et al., 1984). Sex steroids in pre- and post-natal mammals have been shown to clearly activate nervous centers which influence or program certain behaviors that occur in later life stages (Van Tienhoven, 1983). Therefore, exogenous administration of low doses of estrogens in salmonids during critical developmental stages may have long-term detrimental effects which are not immediately assessable on their various behaviors or reproductive development.

Precocious male Atlantic salmon become sexually mature while the salmon are still in fresh water prior to their seaward migration. Precocious sexual maturation of males has been known to occur in as much as 20% of natural and hatchery populations of Atlantic salmon (Saunders et al., 1982). In the present study, precocity was noted in 50% of the yearling male fish in January. In April, greater than 70% of the male salmon had regressed testes. In this study, the experimental groups of Atlantic salmon were held under a 12L:12D photoperiod. There were no sexual precocious male Atlantic salmon in the Gaspe stock that was held under a natural photoperiod. The physiological basis of precocious sexual development in males is poorly understood. Photoperiod was shown to influence the onset of ripening in precocious Baltic salmon parr (*Salmo salar* L., Lundquist, 1980). It is likely in the present study that the 12L:12D photoperiod may be partly responsible for the sexual precocity that was noted in the experimental groups of salmon. Interestingly, the administration of estrogens appeared to inhibit the precocious sexual development that was observed in the control fish.

In conclusion, further studies are required to fully evaluate the effects of estrogens on sex reversal and the health of the treated fish and their progeny. Caution must be exerted in the use of exogenous steroids in juvenile salmon, particularly when the fish are in certain critical development stages, since the steroids may have some undesirable long-term effects.

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. Morphol., 5: 225—249.

Baggerman, B., 1960. Factors in the diadromous migration of fish. Symp. Zool. Soc. London I, October 1959, pp. 33—60.

Barber, H.R.K. and Sommers, S.C., 1974. Vaginal adenosis dysplasia, and clear cell adenocarcinoma after diethylstilbesterol treatment in pregnancy. Obstet. Gynecol., 43: 645—652.

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. Effects of estradiol-17β and 17α-methyltestosterone on gonadal differentiation in the coho salmon (*Oncorhynchus kisutch*). Aquaculture, 17: 267—278.

Grollman, A., 1964. Clinical Endocrinology and Its Physiologic Basis. Lippincott, Philadelphia, PA, 442 pp.

Herbst, A.L., Ulfelder, H. and Poskanzer, D.C., 1971. Adenocarcinoma of the vagina: association of maternal stilbesterol therapy with tumor appearance in young women. N. Engl. J. Med., 284: 878—881.

Jalabert, B., Billard, R. and Chevassus, B., 1975. Preliminary experiments on sex control in trout: production of sterile fishes and simultaneous self-fertilizable hermaphrodites. Ann. Biol. Anim., Biochim., Biophys., 15: 19—28.

Johnstone, R., Simpson, T.H. and Youngson, A.F., 1978. Sex reversal in salmonid culture. Aquaculture, 13: 115—134.

Johnstone, R., Simpson, T.H. and Walker, A.F., 1979. Sex reversal in salmonid culture. Part III. The production and performance of all-female populations of brook trout. Aquaculture, 18: 241—252.

Lundquist, H., 1980. Influence of photoperiod on growth in Baltic salmon (*Salmo salar* L.) with special reference to the effect on precocious sexual maturation. Can. J. Zool., 58: 940—944.

Nakamura, M., 1981. Feminization of masu salmon, *Oncorhynchus masou*, by administration of estradiol-17β. Bull. Jpn. Soc. Sci. Fish., 47: 15—29.

Saunders, R.L., Henderson, E.B. and Glebe, B.D., 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture, 28: 211—229.

Serra, G.B., Villani, L. and Panici, P.B., 1983. Ovarian tumors. In: G.B. Serra (Editor), The Ovary. Raven Press, New York, NY, pp. 401—419.

Simpson, T.H., Johnstone, R. and Youngson, A.F., 1976. Sex reversal in salmonids. Int. Counc. Explor. Sea CM/E: 48, 6 pp.

Sower, S.A., Schreck, C.B. and Evenson, M., 1983. Effects of steroids and steroid antagonists on growth, gonadal development, and RNA/DNA ratios in juvenile steelhead trout. Aquaculture, 32: 243—254.

Sower, S.A., Sullivan, C.V. and Gorbman, A., 1984. Changes in plasma estradiol and effects of triiodothyronine on plasma estradiol during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 54: 486—492.

Van Tienhoven, A., 1983. Reproductive Physiology of Vertebrates. Cornell University Press, Ithaca, NY, 491 pp.

Yamazaki, F., 1983. Sex control and manipulation in fish. Aquaculture, 33: 329—354.