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Hormones and Sex Control in Fish with Particular Emphasis on Salmon[1]

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Abstract

The application of sex control in finfish aquaculture offers significant benefits for the optimization of production strategies and for the reproductive containment of farmed fish. This paper presents a review of methods developed in salmon for the direct endocrine control of sex differentiation and the indirect control of sex through the integration of endocrine and genetic technologies.

Introduction

The control of sex differentiation has become an important and, in some cases, essential element in the culture of several coldwater and tropical teleosts. Many fish are sexually dimorphic with regard to important production characteristics such as growth rate, age at sexual maturity, maximum size and external appearance. On the other hand, sterile fish never undergo sexual maturation and may live longer. Already several species of salmonids and tilapia are grown in monosex culture, and, as aquaculturists strive to improve production efficiency, we can expect additional species to be grown in monosex male or female culture or as sterile fish.

In addition to the culture of sex-controlled fish for their production characteristics, interest has been growing recently in sterilization as a means to facilitate the use of genetically modified fish for aquaculture purposes by preventing reproduction with wild conspecifics in the event of an escape.

The production of monosex or sterile stocks can be achieved either by direct endocrine intervention through treatment with natural or synthetic gonadal steroids, especially androgens and estrogens; or by a combination of endocrine and genetic techniques to produce monosex gametes which are in turn used to generate monosex offspring. Examples include direct masculinization of tilapia larvae with a dietary androgen such as 17α methyltestosterone, production

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of monosex female chinook salmon by fertilization of ova with sperm from androgen-treated genotypic female salmon, and production of sterile Atlantic salmon by the generation of monosex female triploids.

In this paper we review the current status of sex control in fish utilizing salmonids as the model. We present an overview of the application of sex control in salmonid aquaculture, the endocrine control of sex differentiation, and the integration of endocrine with genetic technologies.

Sex Control in Salmonid Culture

The culture of salmon in seawater pens has undergone rapid growth during the last decade in several Pacific Rim countries and in Europe, and at present, worldwide production is still increasing. In Atlantic waters the culture of Atlantic salmon (*Salmo salar*) predominates. In the Pacific, the culture of chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) has predominated, but there is now increased interest in the culture of Atlantic salmon. In the cold freshwaters of the world, rainbow trout (*Oncorhynchus mykiss*) is the predominant cultivated salmonid.

The choice of species is very much related to production efficiency and product characteristics. The aquaculturist rearing salmonids seeks a species or strain that can be cultivated at high density, grows rapidly, has a good food conversion efficiency, good external appearance and flesh quality, and is disease resistant. In addition, the rearing of salmon in sea pens requires fish that transfer easily to seawater and grow efficiently to a size of 4 kg. Sexual phenotype is an important factor in several of the above characteristics. Thus in each species, male salmon mature earlier on average than female salmon and, as a consequence, reach a smaller average size. The onset of sexual maturation in both sexes leads to rapid deterioration of both external appearance (loss of silvering) and flesh quality (color, texture, lipid and protein content). Sterile fish do not undergo any of the changes associated with sexual maturation and thus remain in prime market condition during the reproductive season.

Each of the cultivated salmonids has a characteristic life cycle and an associated production cycle which is amenable to improvement through the application of sex control technologies. The aquaculture production of monosex female chinook salmon in British Columbia currently exceeds the total commercial catch of wild and hatchery produced chinook on the Pacific Coast of Canada. The growth in chinook salmon production to Can$ 80 million at first sale in 1991 was closely linked to the development and implementation of sex control methodologies. Male chinook mature at 2, 3 or 4 years of age, while females mature on average one year later at 3, 4 and 5 years. The culture of monosex female chinook salmon therefore provides the salmon farmer with a longer period in which to grow and harvest this species before sexual maturation and the associated decline in marketability ensues.

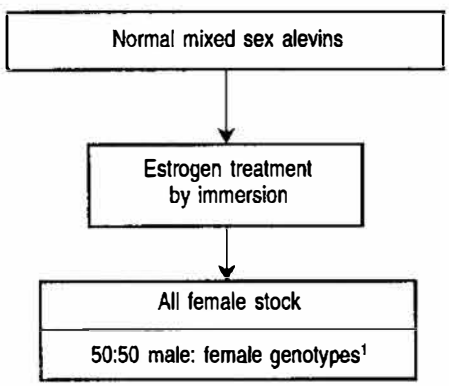
The Atlantic salmon is the major cultivated salmon in the world and is being grown in increasing numbers in Pacific Rim countries in addition to several European countries. Environmental and genetic factors result in a variable proportion of Atlantic salmon reaching sexual maturity as grilse after one sea

winter, a year earlier than full-sized salmon. Grilse are smaller and command a lower value per kilogram than salmon. The occurrence of grilse in significant numbers can therefore have serious economic impact on salmon cultivation. There are usually a greater proportion of male than female grilse. The production of monosex female Atlantic salmon would thus reduce but not eliminate the occurrence of grilse. A second alternative for Atlantic salmon is to utilize sterile fish produced through the generation of monosex female triploids. Extensive trials have been conducted in Scotland on the implementation of this technology (Johnstone et al. 1991).

Hormonal Feminization

Phenotypic all-female populations can be produced by direct treatment with natural or synthetic estrogens (Fig. 1). The critical variables are time of initiation of treatment, duration of treatment, choice of estrogen and dose. In Pacific salmon, studies on the effect of single immersion treatments have enabled the delineation of the period during ontogenesis when sexual phenotype is labile and most sensitive to the influence of external estrogen treatment. This labile period occurs in coho salmon prior to first feeding (Goetz and Donaldson 1979), just before, during and shortly after hatching (Piferrer and Donaldson 1989). Recently we examined the effect of single treatment duration on the response of ultimate sexual phenotype to treatment with estradiol 17β (E_2) and the potent synthetic estrogen ethynylestradiol-17α (EE_2). At 10°C we achieved 100% feminization at a 400 $\mu g \cdot L^{-1}$ dosage level after 8 h with E_2, and after 2 h with EE_2 (Piferrer and Donaldson 1992). These single treatments did not inhibit growth but resulted in modifications of ovarian morphology in up to 40% of the fish.

While direct feminization with estrogen can be utilized after appropriate regulatory approval to produce a phenotypic female population for production purposes, the use of ova from such fish for breeding purposes would result in an increased proportion of males in the offspring.



[1]Not suitable as broodstock

Fig. 1. All-female production by direct feminization.

Hormonal Masculinization

Direct masculinization via androgen treatment can be utilized to generate phenotypic all-male populations as in tilapia, or as a necessary step in the indirect generation of monosex female populations in species where the female is homogametic, through the use of monosex female sperm from phenotypic males with female genotypes (Fig. 2). In salmon, the labile period for masculinization is similar to that for feminization (Piferrer and Donaldson 1989). While 17α methyltestosterone is commonly used as a potent masculinizing agent, there have been occasions when paradoxical feminization has occurred (Solar et al. 1984; Piferrer and Donaldson 1991). We have utilized monosex female chinook salmon embryos to compare the masculinizing potency of natural and synthetic, aromatizable and non-aromatizable androgens, and found that paradoxical feminization did not occur when the non-aromatizable androgens, 11 ketotestosterone and 17α methyldihydrotestosterone, were used (Piferrer et al. 1993). This latter compound was particularly potent and was able to convert the monosex female embryos into 100% phenotypic males after a single 2-h treatment at a low dosage.

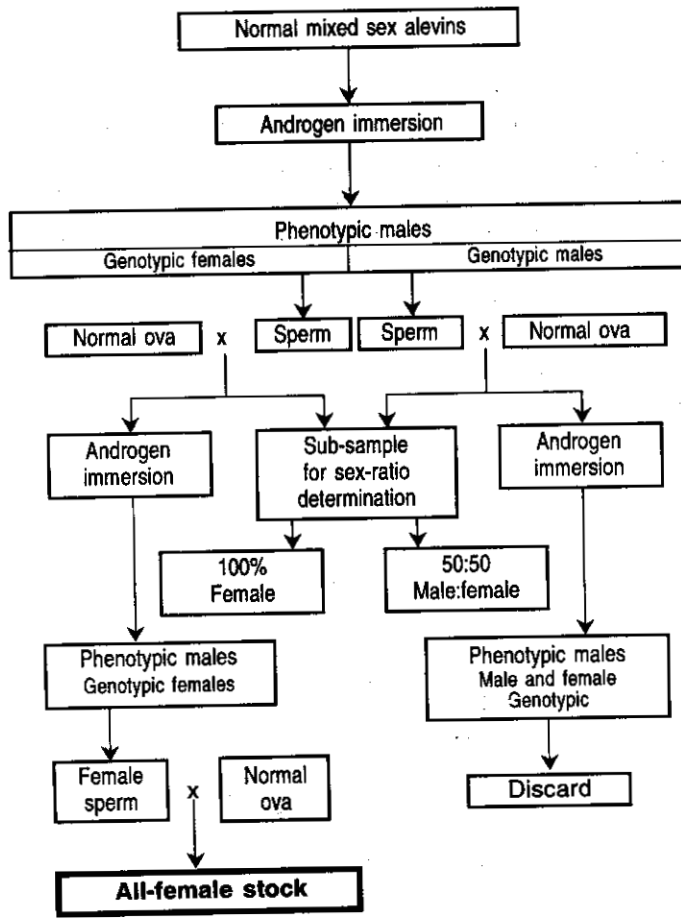


Fig. 2. Direct masculinization and indirect monosex all-female production by masculinization and progeny testing.

Hormonal Sterilization

Sterility can be induced by treatment with androgen at higher dosages and for longer periods than is required for masculinization (Goetz and Donaldson 1979; Donaldson et al. 1991; Piferrer et al. 1994). This technique has been tested on coho and chinook salmon but is not currently utilized as a production technique.

Integration of Hormonal and Genetic Techniques

Endocrine and genetic techniques have been integrated for the production of both monosex and sterile fish. The most utilized sex control technique to date in salmonid culture has been the use of masculinized genotypic females to produce monosex female sperm. Initially, this was a two-generation technique as progeny testing was required to identify phenotypic males which had a female genotype. Now two new techniques are available for the production of monosex female sperm in a single generation.

First, as female salmonids are homogametic (Hunter et al. 1982), the induction of gynogenesis results in the production of female embryos. If these are then masculinized during sex differentiation by androgen treatment, the resultant fish are phenotypic male gynogens, and these will produce only X-chromosome bearing sperm at maturity (Fig. 3). We have recently demonstrated the use of this technique for coho and Atlantic salmon.

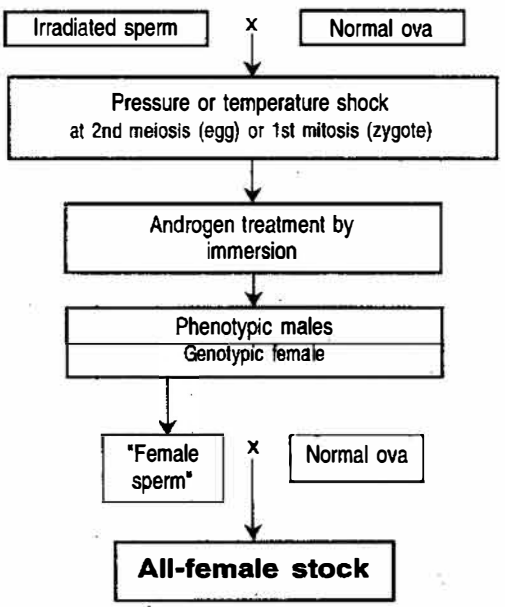


Fig. 3. All-female production by gynogenesis and masculinization.

The second technique involves masculinizing embryos of mixed sex, and then utilizing a Y-specific DNA probe (Devlin et al. 1991) to directly distinguish genetic males from genetic females. The latter are retained and will produce female sperm at maturity (Fig. 4).

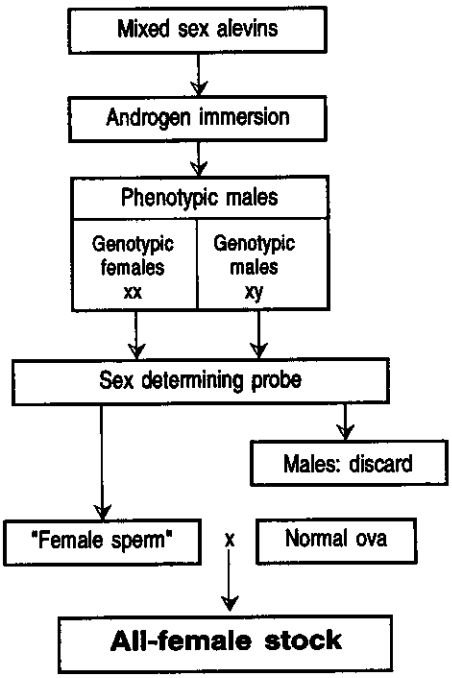


Fig. 4. All-female production by masculinization and Y specific DNA probe.

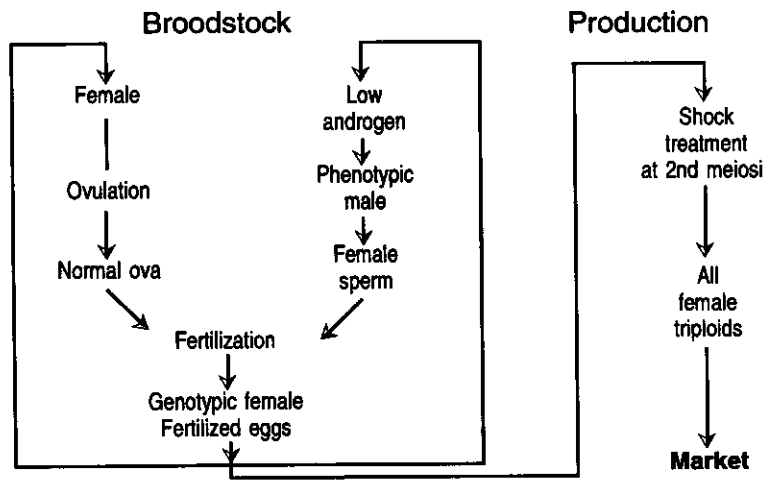


Fig. 5. Production system for the generation of triploid monosex female salmonids (modified from Donaldson and Benfey 1987).

Another technique which combines endocrine and genetic manipulation is the production of monosex female triploid salmonids (Fig. 5). This technique is based upon the fact that gonadal development is suppressed to a much greater extent in female triploids than in male triploids (Benfey and Donaldson 1988).

The future development of cloning techniques to produce populations of identical gynogenetic fish for culture will require the use of hormonal sex control to generate phenotypic males which can then be utilized to fertilize ova produced by the female clones, thus perpetuating the clone (Fig. 6).

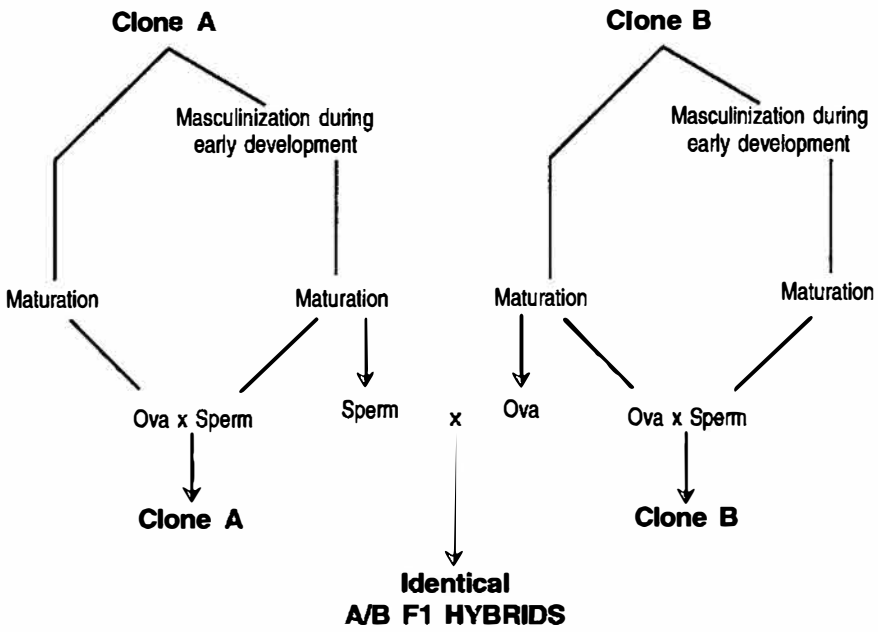


Fig. 6. Options for propagation of and from clonal lines.

Reproductive Containment of Genetically Modified Fish

The future application of transgenic fish in aquaculture (Hew and Fletcher 1992) is expected to be closely regulated. It is generally accepted that for the foreseeable future, only sterile transgenics will be permitted for culture purposes (Devlin and Donaldson 1992). The most feasible method for sterilizing transgenics is the production of monosex female triploids. The issue then becomes whether total sterility can be guaranteed, thus preventing reproductive interaction between escaped transgenic fish and wild conspecifics.

Conclusions

As aquaculturists strive to improve production efficiency and maximize product quality, we can expect increased application of sex control technologies. The trend will be toward integration of endocrine and genetic techniques to produce monosex gametes, thus avoiding the use of steroids in production fish.

The future implementation of transgenic fish in aquaculture will necessitate the use of reproductive containment.

References

Benfey, T.J. and E.M. Donaldson. 1988. Triploidy in the culture of Pacific salmon. Proceedings of the Aquaculture International Congress: 549-554.

Devlin, R.H., B.K. McNeil, T.D.D. Groves and E.M. Donaldson. 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 48: 1606-1612.

Devlin, R.H. and E.M. Donaldson. 1992. Containment of genetically altered fish with emphasis on salmonids. In: Transgenic fish (eds. C.L. Hew and G.L Fletcher), pp. 229-265. World Scientific, Singapore.

Donaldson, E.M. and T.J. Benfey. 1987. Current status of induced sex manipulation. Proceedings of the Third International Symposium of Reproductive Physiology in Fish: 108-119.

Donaldson, E.M., F. Piferrer, I.I. Solar and R.H. Devlin. 1991. Studies on hormonal sterilization and monosex female technologies for salmonids at the West Vancouver Laboratory. Canadian Technical Report of Fisheries and Aquatic Sciences 1789: 37-45.

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

Hew, C.L. and G.L. Fletcher. 1992. Transgenic fish. World Scientific, Singapore. 274 pp.

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

Johnstone R., H.A. McLay and M.V. Walsingham. 1991. Production and performance of triploid Atlantic salmon in Scotland. Canadian Technical Report of Fisheries and Aquatic Sciences 1789: 15-36.

Piferrer, F. and E.M. Donaldson. 1989. 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 E.M. Donaldson. 1991. Dosage dependent differences in the effect of aromatizable and nonaromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). Fish Physiology and Biochemistry 9: 145-150.

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

Piferrer, F., I.J. Baker and E.M. Donaldson. 1993. Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). General and Comparative Endocrinology 91: 59-65.

Piferrer, F., M. Carrillo, S. Zanuy, I.I. Solar and E.M. Donaldson. 1994. Induction of sterility in coho salmon, *Oncorhynchus kisutch*, by androgen immersion before first feeding. Aquaculture 119: 409-423.

Solar, I.I., E.M. Donaldson and G.A. Hunter. 1984. Optimization of treatment regimes for controlled sex differentiation and sterilization in wild rainbow trout (*Salmo gairdneri* Richardson) by oral administration of 17α-methyltestosterone. Aquaculture 42: 129-139.

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