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**Aquaculture Collaborative Research and Development Program (ACRDP) Fact Sheet**

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# Production of All-Female Populations of Coho Salmon, *Oncorhynchus kisutch,* using Y-Chromosomal DNA Markers

## Summary

Some strains of coho salmon show moderate to high levels of early sexual maturation, at a sub-optimal market size. Development of all-female strains of this species could be of significant benefit for the aquaculture industry, by eliminating losses arising from early maturation of males, and allowing for a doubling in roe production from the same number of production animals as in mixed sex cultures. Single sex populations also provide an environmental benefit in areas where non-native species are being cultured, by providing a highly effective method for reproductive containment.

Application of simple PCR-based diagnostics can allow regular XY males to be distinguished from masculinized XX males, facilitating the development and maintenance of monosex, all-female strains.

## Introduction

Males of some strains of salmon can become sexually mature early, at a sub-optimal market size. To alleviate this problem, all-female strains have been developed in Chinook salmon (*Oncorhynchus tshawytscha*) and now make up the bulk of production of that species in Canada.

Salmonids possess an XY genetic sex determination system which is highly stable, allowing production of pure populations of XX individuals for production purposes. Historically, this has been achieved by coupling sex reversal protocols with family selection. Mixed sex progeny are treated with androgen at the alevin stage which masculinizes XX females into functional males, yielding groups containing both XX and XY males.

The two types of males have been distinguished from one another by:

1) test crossing each individual with regular females, and retaining those which yield only female progeny (this takes many years to accomplish),

2) using distinguishing characteristics (i.e., lack of a sperm duct) that exist between XX and XY males (inducing this characteristic requires lengthy dietary treatments with androgen and is not always reliable),

3) identification of hermaphrodite gonads, which is strongly indicative of an XX genetic background (but only occurs in a small proportion of sex-reversed fish and thus is also unreliable), and

4) the use of Y-chromosomal DNA markers which can distinguish males of XX and XY genotype (this is very reliable).

Coho salmon, another species of commercial importance (although less so in Canada than Chinook or Atlantic salmon) also show moderate to high levels of early maturation in some strains. In addition, the roe of this species is highly valuable. Development of all-female strains would be of significant benefit for aquaculture.

## Methods

The steps involved in producing an all-female strain of salmon are (Figure 1): 1) masculinize the first generation; 2) develop a genetic marker that will distinguish genetic males (XY) from genetic females (XX); 3) test each fish with the genetic marker and remove all the genetic males (XY); 4) verify that the removed fish are really males (XY) by growing them to maturity and mating them with normal females (XX) to produce a mixture of male (XY) and female (XX) offspring (used only for research projects); and 5) mating the screened-in fish which are genetically female (XX - but appear to be males and produce sperm) with normal females (XX) to produce all female (XX) offspring.

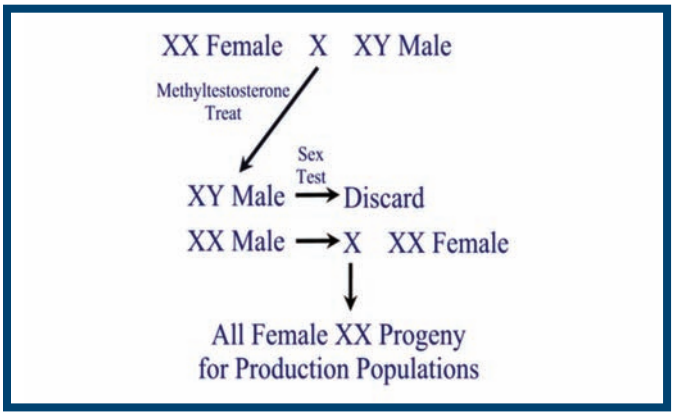


**Figure 1.**
*Schematic of process to create an all-female strain using masculinization and genetic screening.*

### *Masculinization*

Coho salmon were masculinized by treating newly-hatched fish (alevins) with methyl-testosterone and methyldihydro-testosterone, producing fish of mixed genetic make-up (XX and XY) that only have male sexual characteristics. In a production setting, these fish can be genotyped and the genetic males discarded, saving rearing costs; however, screening usually occurs at the smolt stage or even later. Some operators do not screen them out, choosing to select XX males at sexual maturity. In this study, these fish were raised in freshwater until smolting and in sea pens to maturity. Broodstock were transferred to a fresh water site, individually tagged, and blood samples taken for genotyping.

### *Development of Genetic Marker*

Determination of genetic sex (XX vs XY) was performed using a genotyping test designed to detect the coho salmon Y chromosome. This assay is based on the presence of a growth hormone pseudogene (GH-P) on the Y chromosome which can be distinguished from other autosomal copies of GH genes using simple Polymerase Chain Reaction (PCR)-based diagnostics. The PCR test utilized primers GH5 and GH6 which span intron E of all GH genes in the genome. Polymerase chain reactions were performed using annealing

temperatures between 50°C and 60°C, and products were electrophoresed in 0.8% agarose gels stained with ethidium bromide. Oligonucleotide sequences used as amplification primers are:

**GH5: 5'-AGCCTGGATGACAATGACTC-3';**
**GH6: 5'-CTACAGAGTGCAGTTGGCCT-3'.**

The second step was to develop a genetic test that could reliably identify the Y chromosome in fish blood or tissue, thereby marking which fish were genetic males (XY) and which were masculinized females (XX).

The Y-chromosomal marker (GH-P) previously found to be closely linked to the sex determination locus in wild coho salmon was also found to be Y-linked in the domesticated population (unpublished data). Thus, sex genotype can be readily determined by application of a simple PCR diagnostic which can be performed using crude tissue or blood preparations. The GH primers used for amplification yield 2 products in genetic females (derived from GH1 and GH2), whereas in males these fragments plus a 290 base pair band derived from the Y-linked GH pseudogene are produced (Figure 2). The assays can be rapidly conducted such that under ideal conditions, data can be generated and returned to the broodstock site within a single day.

### *Genetic Screening*

Following determination of genetic sex, genetic females (XX) were selected and examined for evidence of masculinization (milt production and/or morphological characteristics). If produced, sperm were collected directly from sperm ducts. All other genetic females were euthanized and their gonadal tissue examined for signs of testicular development, in which case milt was collected by gentle maceration of excised testicular tissue. The collected milt was then used in crosses with regular female (XX) broodstock and progeny reared in fresh water until pre-smolt stages. Progeny were sampled and also subjected to sex marker genotyping to verify their monosex condition.

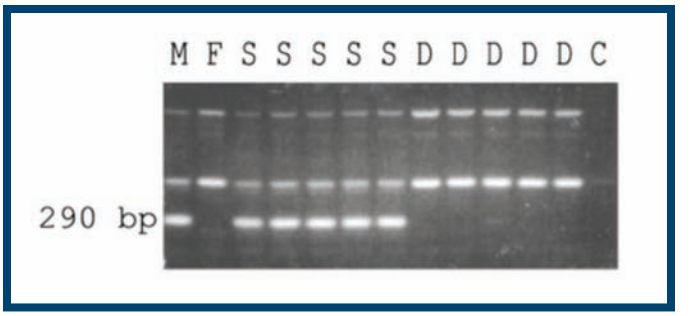


**Figure 2.**
***Agarose gel showing sex-specific PCR amplification of the Y-linked GH-P fragment (290 bp) in coho salmon. M, mother; F, father; S, sons; D, daughters; C, no template control.***

## Results

Over a five year period, coho salmon were masculinized with methyltestosterone and reared to sexual maturity for use as broodstock. At maturation, blood was drawn and genotyped for genetic sex (Table 1).

**Table 1.**
***Production of broodstock and monosex progeny.***

| Brood year | 1999 | 2000 | 2001 | 2002 | 2003 |
|---|---|---|---|---|---|
| Brood tested | 522 | 591 | 582 | 158 | 210 |
| % masculinated | 1% | 4% | 33% | 41% | 86% |
| % nonmaturing | 9% | 38% | 55% | 41% | 14% |
| Number of progeny smolts tested | 1600 | 1600 | 1600 | 600 | 600 |
| Progeny % female | 100% | 95% | 100% | 100% | 100% |

Phenotypic males of female (XX) genotype were selected for use as broodstock and used in crosses with regular females. In the first year of production, a high proportion of females were obtained in progeny derived from such crosses (Table 1). Exceptional males were recovered in the second year, and these were tested and found to contain a Y chromosome. Thus exceptional males did not

appear to be arising from autosomal genetic effects or environmental influences, but rather most likely arose by the accidental inclusion of XY brood males into production crosses. In subsequent years, 100% female progeny have been observed, demonstrating the utility of this approach in the production of monosex populations for this species.

In addition to benefits of monosex strains derived from elimination of sexual maturity, all-female strains also allow for enhanced roe production which can be of benefit if appropriate markets have been developed. Thus, for coho salmon, the use of monosex female strains has allowed a doubling in roe production from the same number of production animals previously used in mixed sex culture (Figure 3).

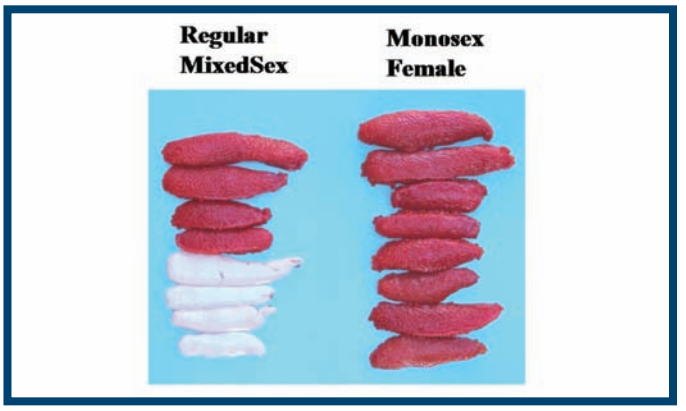


**Figure 3.**
*Increased roe production from monosex population.*

## Conclusions

While the primary purpose of monosex strain development for aquaculture is to enhance production, in some cases single sex populations may provide an environmental benefit as well. For example, in areas where non-native species are being cultured, the use of single sex populations, if adopted universally, can provide a highly effective method for reproductive containment. Such fish may live out their life following escape, but with no conspecifics in nature of the opposite sex with which to breed, their direct impact would be limited to a single generation and would be expected to be of limited magnitude. Monosex strains also have utility in the production of nonreproductive populations of triploid fish. For many species, triploid males still undergo sexual maturation and produce functional although aneuploid sperm, whereas females do not show significant ovarian development. Thus, all-female triploid populations are advantageous for purposes of reproductive containment and for suppression of sexual maturation.

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*For further information on this and other ACRDP projects, visit:* ***http://www.dfo-mpo.gc.ca/science/aquaculture/acrdp-pcrda/main_e.htm***

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