A Review Of Methods For Obtaining Monosex Fish And Progress Report On Production Of Monosex White Amur[1]

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ABSTRACT

A theoretical basis for the production of monosex white amur (*Ctenopharyngodon idella* Valenciennes) for aquatic weed control was developed. Gynogenesis was observed in white amur eggs treated with UV-irradiated milt from carp (*Cyprinus carpio* L.) and progeny were monosex females. The low percentage yield in gynogenesis limits this method of monosex production to species with high fecundity. Androgenesis occurred in carp eggs fertilized with non-irradiated white amur milt. One of the expected genotypes (YY) in androgenesis might be useful for production of only male fish. Breeding YY males to normal XX females should give only XY male progeny. Sex reversal techniques have been used to produce monosex female fish. In the method proposed here, gynogenetic white amur were administered androgen in an attempt to reverse genetic females into functional males. The resulting sex-reversed fish would be capable of siring all-female broods.

INTRODUCTION

Fish exclusively of one sex would be useful for testing the environmental effects of an exotic species on native flora and fauna without the possibility of naturalization. The white amur is a herbivorous fish from Asia that is being evaluated for use as a control agent of aquatic weeds in the United States. Previous work suggests that production of monosex white amur is possible (11, 12). The objective of this paper is to report progress of applying monosex culture methods to white amur and to review methods that have yielded monosexes in other fishes.

METHODS AND MATERIALS

Broodfish carp and white amur were maintained in freshwater ponds on a diet of natural food supplemented with pellets. Fish were handled only during May or June at the time of artificial spawning. Broodfish were seined in the early morning to minimize thermal shock, and transported to the laboratory in well-oxygenated water to which was added the anesthetic quinaldine (2-methylquinoline) at 50 mg/l. Indoors they were held in 680-liter tanks covered with netting in running water at 24 C. They were handled only under anesthetic during injections and spawn taking.

Egg and milt maturation was induced by hormone injections. Males of both species were given acetone-dried carp pituitary at 2 mg/kg of body weight 12 hr prior to milt need. Female carp received one 10 mg/kg injection of dried carp pituitary. Female white amur had three injections; on day one 400 IU/kg of human chorionic gonadotropin, on day two 1600 IU, and on day three 10 mg/kg of dried carp pituitary. Ovulation in carp and white amur, occurred about 12 hr after the pituitary injection and eggs were stripped within 1 hr of ovulation.

White amur eggs were incubated in 250 *l* fiberglass pots 76 cm in diameter, 76 cm deep with a 45° conical bottom. Water was introduced at the bottom of the conical bottom at about 2 liters per min and discharged through a pipe on the side near the top of the pot. Newly hatched fry swam out the side pipe and were collected in a screen box. Each pot held up to 300,000 eggs and 10 pots were used. Carp eggs were incubated in baskets (12x20x10 cm) made of 0.5mm-mesh saran screening. The baskets were partially submersed in water in an aluminum trough. Incubation temperature was 24 C and oxygen was 8.0 mg/*l*.

Two days after hatching fry were fed finely-divided boiled egg yolk and nauplii of brine shrimp (*Artemia salina* Leach). At 7 days fry were either stocked in 0.04 ha ponds or placed on experimental diets.

Gynogenesis was induced by treating 11 million white amur eggs taken from 41 females with irradiated milt from carp. Milt was irradiated after mixing with 4 times its volume of ice-cold Hanks' balanced salt solution without bicarbonate. For irradiation milt was placed in 9 cm diameter petri dishes and oscillated at 50 cycles/min under a UV lamp of wavelength 254 nm and a power of 6.5 mW/cm^2 (for 10 min). Diploid gynogenetic fish were separated from haploids because the latter were incapable of swimming out the hatching pot.

Androgenesis occurred spontaneously in carp egg fertilized with non-irradiated milt from white amur. There were 160,000 eggs from 15 carp treated with white amur milt.

Sex reversal was attempted and design of the study is reported here; final results are not yet available. Gynogenetic white amur were fed methyltestosterone [17(α)-methyl-Δ^4-androsten-17-(β)-ol-3-one]. Feed containing this androgen was made by mixing 40 ml of alcohol-androgen solution with 100 g of Fish and Wildlife Service formula No. 3 feed. The alcohol was subsequently evaporated by air-drying. Before feeding to young fry the feed was ground with a mortar and pestle. A total of 1007 gynogenetic white amur were fed methyltestosterone at a dose of 30 mg/kg

[1]This study was supported by funds from the U. S. Army Corps of Engineers.

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of feed and 78 were given 60 mg/kg of feed. There were 387 gynogenetic white amur which received a control diet made exactly the same except the hormone was omitted. In addition 800 other gynogenetic white amur were stocked directly into ponds at 7 days of age. The age at which hormone feeding was initiated and the duration of the feeding period is given in Table 1.

GYNOGENESIS

About 9 million white amur eggs developed gynogenetically and several million larvae hatched. The vast majority never attained the power of locomotion and perished in the pots. About 40,000 gynogenetic fish swam out of the pots and subsequently grew into fish which appeared in every detail of morphology, biochemistry and chromosome number to be white amur (to be reported in detail elsewhere).

Monosex progeny were expected in gynogenesis because only female chromosomes would be present. This expectation is based on the assumption that sex of white amur is primarily determined by inheritance of two X chromosomes from the female parent. All gynogenetic white amur examined to date have been females (also to be reported in detail elsewhere). Gynogenetic carp also are exclusively females (5). However, some species have sex determined by WZ for female and ZZ for male and are expected to have both sexes among gynogenetic progeny. Gynogenetic plaice [*Pleuronectes platessa* (L.)] indeed are both males and females (8). Thus, use of gynogenesis to produce monosexes is limited to those species with XX females and XY males.

Not all species are likely to yield gynogenetic progeny, although it has been reported in a number of fish species (1,2,7,9,12). Also, the percentage yield may limit the practicality of this method. In white amur gynogenesis is a feasible method because of the high fecundity. In species with a limited number of eggs production would be too meager for use in testing exotic species.

TABLE 1. NUMBERS OF GYNOGENETIC GRASS CARP FED METHYLTESTERONE (30 MG/KG OF FEED) FOR DIFFERENT PERIODS AND AT DIFFERENT AGES IN AN ATTEMPT TO REVERSE THE SEXES FOR PRODUCTION OF MONOSEX POPULATIONS. CONTROL FISH WERE FED EQUAL AMOUNTS OF UNTREATED FOOD.

Year	Age (weeks)[a] Start	End	Number stocked in ponds Control	Methyltestosterone
1973	1	10	12	10
	4	14	7	5
1974	1	3	50	50
	1	5	50	50
	1	7	18	4
	4	8	70	140
	4	8	800[b]	626
	6	8	35	33
	6	10	35	29
	11	15	35	26
	16	20	35	34
		Totals	1147	1007

[a] The age at beginning and end of feeding period; fish were stocked into ponds immediately after treatment.
[b] Controls not fed; stocked directly in ponds.

ANDROGENESIS

Androgenesis, development in which the embryo contains only paternal chromosomes due to failure of the egg nucleus to participate in fertilization, might be used for production of only male fish. Reports of androgenesis in fish are limited to haploids produced by irradiation of eggs (6,7,9). Androgenesis was observed in three groups of carp-amur hybrids in which the progeny appeared to be diploid. Of 160,000 eggs 43 androgenetic white amur were produced. Since diploid androgenesis has not previously been reported there is no indication of how it might occur. Perhaps fertilization was with two spermatozoa with subsequent loss of oocyte chromosomes.

On the basis of classical sex inheritance mechanisms, in which two X sex chromosomes result in female and an X and a Y chromosome results in males, the three genotypes of androgenetic progeny depend on the particular sex chromosome carried by each of the two spermatozoa involved in the fertilization. A female would result from the fertilization of an egg by two X-bearing spermatozoa; a male from fertilization by an X-bearing and a Y-bearing spermatozoa. Breeding of the YY male with a normal XX female should produce only male progeny. If fertilization is random, 25% of the progeny should be XX, 50% XY, and 25% YY (Punnett square). Because there is no apparent means of distinguishing the YY from the XY genotype a test cross is required. In testing, each androgenetic male is mated to a normal XX female. Only YY males will sire all-male broods whereas XY males will produce 50% males and 50% females. After their identification, the YY males can be used to sire monosex populations. The YY genotype is expected in about 11 fish assuming this genotype is fully viable, as it is in killifish, *Oryzias latipes* (4) and goldfish (*Carassius auratus*) (15).

SEX REVERSAL

Sex reversal has been used to produce only female goldfish (16) and *Tilapia mossambica* Peters (3). The theory of sex reversal has been reviewed and numerous cases cited in which reversal of sex has been achieved (10, 14). Procedurely, young are fed an androgen to reverse females into apparent males; a test cross is made to distinguish the sex reversed individual from ordinary males. In testing, each male is mated with a normal XX female; sex-reversed males (XX genotype) sire all females. For white amur this procedure would take about 5 years—3 to raise the androgen-fed fish and 2 to rear their progeny. The sex reversal technique proposed here is considerably shorter because the test cross is unnecessary if monosex gynogenetic females are used.

Gynogenetic offspring produced in 1973 and 1974 were fed methyltestosterone (Table 1). Of 1147 fish fed the androgen and 387 fed a control diet, 1007 androgen-fed fish and 347 control fish survived the feeding period and were stocked in ponds. Included in the controls in Table 1 were 800 fry stocked directly in ponds without a laboratory feeding period. Not listed are two groups of fish fed methyltestosterone at 60 mg/kg of feed. One group of 8 fish

received this higher dosage for 9 weeks beginning at 1 week of age in 1973 and another 70 were fed for 4 weeks beginning at 4 weeks of age in 1974. The length of time on treated feed and the age at which feeding began and ended was varied in an attempt to deliver the androgen at the time of sexual differentiation. This range of treatment should provide some sex-reversed fish; this will be determined in 1976 for the 1973 year class and in 1977 for the 1974 year class. Even a few sex reversed males could be used to sire millions of monosex females using ordinary cultural procedures.

CONCLUSION

If weed control with monosex white amur produced by gynogenesis, androgenesis, or sex reversal is to be economically feasible, it must cost less than chemical control or mechanical removal. On the basis of research costs and cultural operations, I estimate that monosex fish can be produced and raised to stocking size for about $1 each. Assuming that 60 fish per ha would control weeds for 6 years (13) the annual cost would be $10/ha, less than 0.1 the cost of chemical or mechanical treatment. Production of monosex white amur should cost only slightly more than the production of normal bisexual fish because special treatment is required only to produce the broodfish. Thereafter, monosex production requires only ordinary cultural procedures.

In my opinion use of monosex white amur is justified because risks associated with the release of this exotic species would be markedly reduced with little change in costs or benefits. Furthermore, monosex fish will assure that unanticipated damage would be only temporary. By using monosex white amur, the operator should be able to manage aquatic vegetation rather closely by adjusting the number of fish released; vegetation-choked water and totally denuded bottoms can both be avoided.

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