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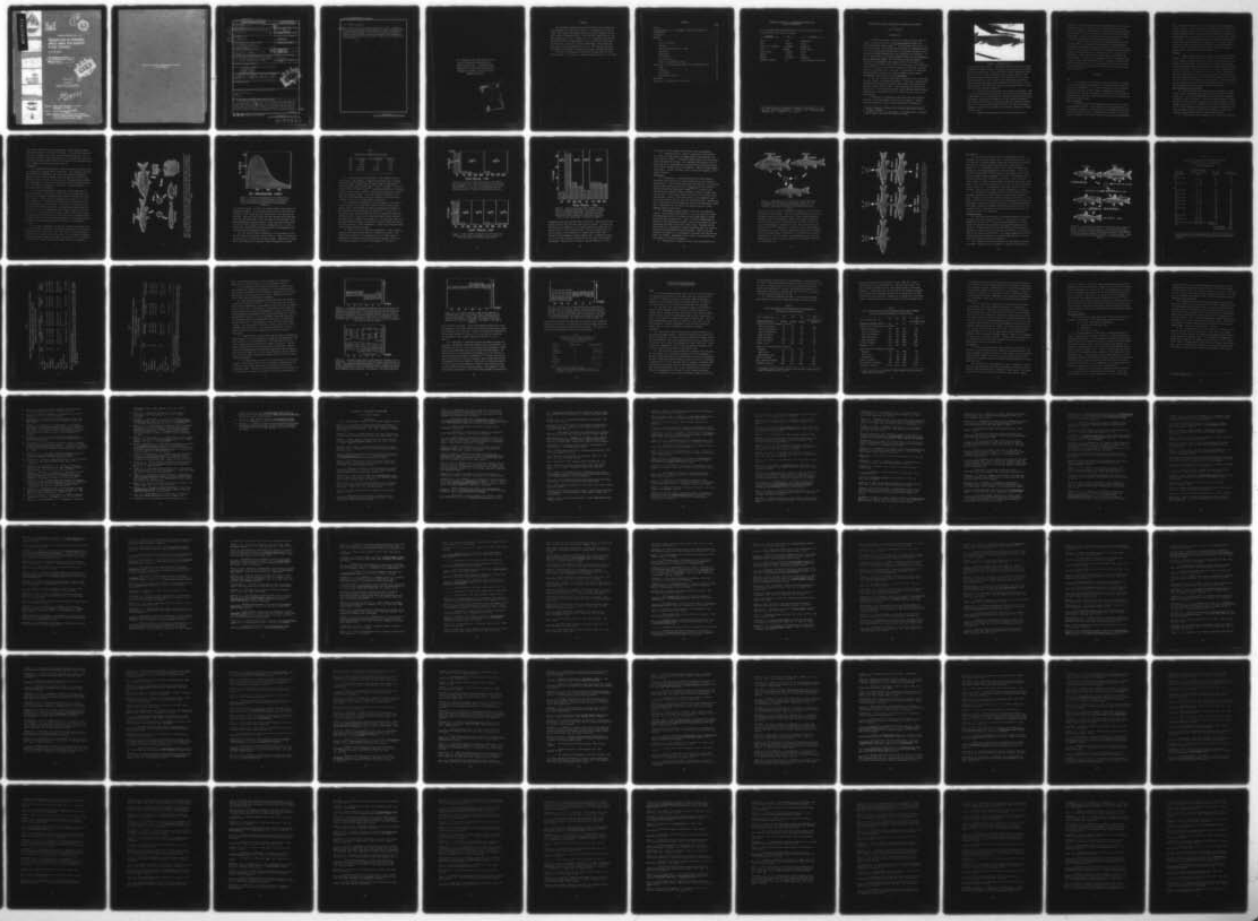

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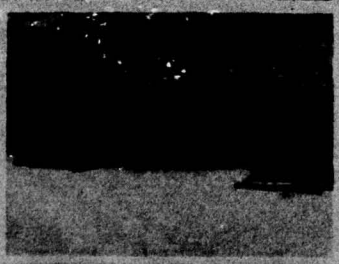

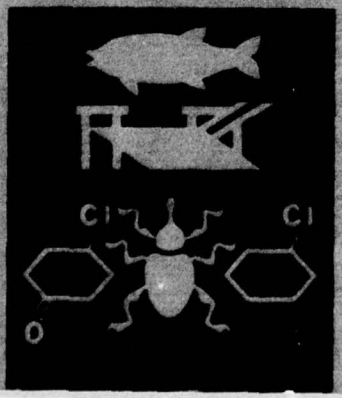

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PRODUCTION OF MONOSEX WHITE AMUR FOR AQUATIC PLANT CONTROL

OCT. 1976

CONTRACT REPORT A-76-1

PRODUCTION OF MONOSEX WHITE AMUR FOR AQUATIC PLANT CONTROL

by Jon G. Stanley

U. S. Department of Interior
Fish Farming Experimental Station
Stuttgart, Arkansas

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The white amur fish has potential for biological control of vegetation in waterways, lakes, and reservoirs. However, intensive and widespread use of white amur should be avoided until it is certain that the fish will not become a naturalized pest. The purpose of the research reported herein was to determine the feasibility of producing populations of white amur which could not reproduce. Gynogenesis, androgenesis, and sex reversal procedures

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were studied as methods for making populations of one sex. Analysis of morphological and biochemical traits showed that monosex white amur could be produced and that these fish are identical to female fish produced by usual fertilization methods. A model for the costs of an operational hatchery facility and weed control program suggested that treatment cost with monosex amur would be $150 per acre in 1975-77, but only about $2 per acre after 1977.

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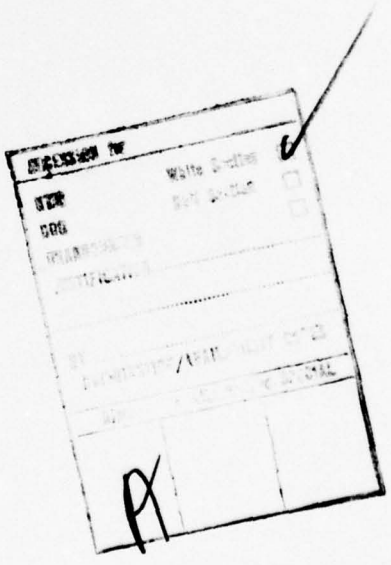

Preface

The information presented herein was performed in part under contract agreement No. APCP-3-73-1 with the U. S. Department of Interior (USDI), Fish Farming Experimental Station, Stuttgart, Arkansas, for the Office, Chief of Engineers (OCE). This study was conducted and the report prepared by Dr. Jon G. Stanley. Dr. E. O. Gangstad, OCE, was the Contracting Officer's representative for the contract; his assistance and constructive criticism is hereby acknowledged. The Mobility and Environmental Systems Laboratory of the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi, monitored the report.

Director of WES during the preparation and publication of this report was COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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Conversion Factors, U. S. Customary to Metric (SI) Units of Measurement

U. S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

Multiply	By	To Obtain
inches	2.54	centimetres
feet	0.3048	metres
miles (U. S. statute)	1.609344	kilometres
acres	4046.865	square metres
quarts liquid	0.94636	litres
gallons	3.78533	litres
pounds	0.45359237	kilograms
Fahrenheit degrees	5/9	Celsius degrees or Kelvins*

* To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use the following formula: $C = (5/9)(F - 32)$. To obtain Kelvin readings, use: $K = (5/9)(F - 32) + 273.15$.

PRODUCTION OF MONOSEX WHITE AMUR FOR AQUATIC PLANT CONTROL

by

Jon G. Stanley*

Introduction

1. Field and laboratory studies have shown that white amur fish (Figure 1), also called grass carp (Ctenopharyngodon idella), will effectively control aquatic weeds.[1-8] The white amur is an exotic fish imported from Asia. Its fusiform body is adapted for spawning migration up rivers. Specialized serrated teeth in the pharynx are used to shred vegetation, thereby allowing this fish to function as an herbivore. Amur are efficacious in controlling submersed plants such as Potamogeton, Egeria, Elodea, and Najas. This fish shows particular promise in eradication of Myriophyllum and Hydrilla, which are not easily controlled by other methods. They also feed on some species of filamentous algae,[2,3,9,10] thus preventing the development of obnoxious scums that occur after vegetation dies. White amur is ineffective in controlling emergent aquatic plants such as cattails (Typha),[4] water hyacinth (Eichhornia),[5] and alligator weed (Alternanthera).[5]

2. The cost of aquatic weed control using white amur is less than that with chemical or mechanical treatment.[4] Generally a stocking level of 20-50 fish per acre** is required, and currently these fish can be produced for less than $1.00 each. One stocking may be effective for about 6 years.[4] Thus vegetation control can be achieved with an initial investment of less than $50 per acre, or $8 per acre for each year of effectiveness.

3. Objections to releasing the white amur have been raised by officials in State and Federal natural resource agencies. Several states have proposed or enacted regulations against introducing or

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** A table of factors for converting U. S. customary units of measurement to metric (SI) units is presented on page 4.

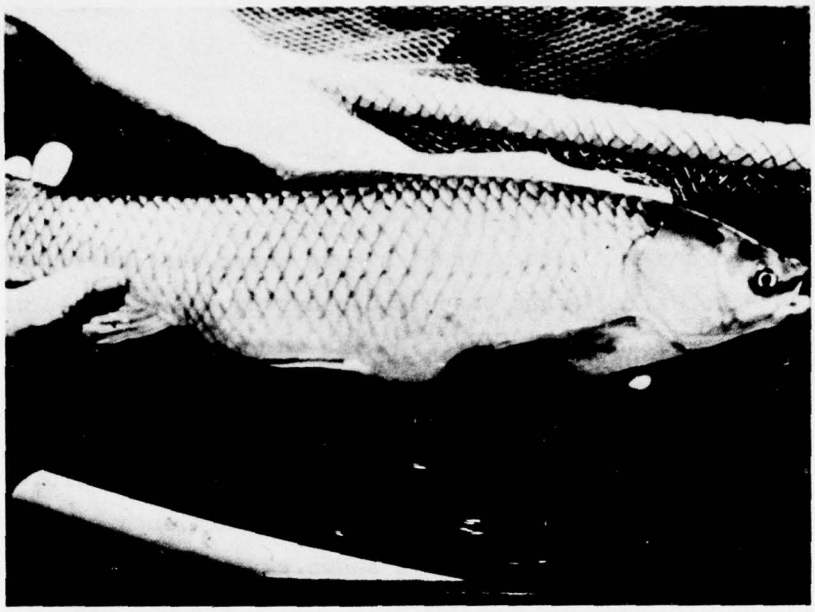

Figure 1. The white amur

stocking this exotic species. Proposed Environmental Protection Agency regulations[11] require a permit for holding and culturing exotic species, including the white amur. Fish and Wildlife Service regulations under the Lacey Act[12] prohibit importation and interstate commerce of the white amur unless a permit is obtained from the Director of the Fish and Wildlife Service. The threat of court injunctions initiated by private conservation groups further inhibits use of this fish. These legal and policy restraints are based on the potential menace this fish poses to native fish and wildlife.[13]

4. The white amur could become a pest in the United States. Some of the same characteristics which make it effective in controlling undesirable weeds in some areas might be a liability should this fish escape to important waterfowl, marsh habitat, or other natural ecosystems.[14] Observations on the effects of white amur have been limited, partially due to the reluctance of waterfowl biologists and other environmental managers to test the fish in habitats vulnerable to disruption through vegetation loss.

5. Competition between native fish and young white amur is

probable. The young of almost all fish, including white amur, feed on zooplankton and insect larvae; adults of many fish species also eat these food organisms. The capacity of white amur for food competition has been shown,[15-17] but not with species of native fish most likely to be affected. Effects of competition could be reduced if larger individuals were stocked and if reproduction could be prevented.

6. Several methods are known for obtaining nonreproducing fish populations.[18] The limitations and potentials of each method were considered, and gynogenesis and sex reversal were selected as the most feasible for application to white amur. Gynogenesis is the development of an egg after penetration by a sperm that does not contribute to inheritance. Since there is no male inheritance, the offspring are totally female. In sex reversal, immature fish are fed androgen (male hormone) to cause male sex differentiation. However, often treatment does not reverse the sex of all fish and thus some females can remain in the population. Sex reversal has been successful in several other species of fish.[19-21]

Research

Aim

7. The objective of this research was to determine the feasibility of producing nonreproducing white amur fish that could be used in aquatic weed management. Use of monosex fish would have an advantage over use of both sexes of amur because environmental risk would be minimized with no decrease in benefits. Monosex amur were achieved by fertilizing amur eggs with irradiated sperm from carp. The methods are described in detail below.

Care of broodfish

8. White amur used as the female parents were at least 3 years old and weighed 12-30 lb. It was possible to obtain eggs from fish in poor physical condition, but fertile eggs were obtained only from fish in good physiological state. To maintain the good health of the fish, they were stocked at less than 20 fish per acre and given supplementary

feed. Broodfish were handled once each year at the time of spawning. Seining was always conducted early in the morning or on cloudy days to prevent temperature shock. The fish were transported to the laboratory in well oxygenated water to which was added an anesthetic--quinaldine (2-methylquinoline). They were held at 73-77°F in 150-gal tanks covered with netting. They were handled only to give injections and take eggs and then only under anesthesia with quinaldine. Carp used to supply sperm were stocked at the rate of 160 fish per acre and fed commercial catfish pellets. Each male carp was used several times during one season, with a 2-week recovery period between uses.

Spawning

9. Eggs and sperm flow from white amur and carp were induced by hormones. Males of both species were injected intraperitoneally 12 hr prior to sperm need with 0.8 mg/lb of acetone-dried carp pituitary suspended in distilled water. One part sperm was mixed with four parts cold Hanks' balanced salt solution without bicarbonate and then refrigerated until needed. Female carp received one 5-mg/lb intraperitoneal injection of pituitary to induce ovulation 12 hr later. Female white amur were administered three injections consisting of 200 IU/lb human chorionic gonadotropin (HCG) on the first day, 800 IU/lb on the second day, and 5 mg/lb of carp pituitary on the third day. Ovulation in both species occurred about 12 hr after the pituitary injection. Fish were anesthetized with 50 ppm quinaldine prior to spawn taking.

Egg incubation and care of young

10. In nature, white amur spawn in the open channels of large rivers. Their semibuoyant eggs float in the river current until the larvae hatch. In artificial reproduction, the process of suspending the eggs in water is simulated. Semibuoyant eggs are generally incubated in 5-qt McDonald jars, cylinders with a hemispherical or conical bottoms, in which water is introduced at the bottom and flows out the top.[22] These incubators were tested but were found unsatisfactory for white amur eggs because of the frequent adjustments of water flow needed to achieve proper rolling of eggs without overflow.

11. In the Soviet Union, incubators up to 50 gal in volume are

used for white amur eggs.[23] The U. S. Fish and Wildlife Service conducted tests in a 55-gal fiberglass container (Fiber Tech Engineering, Santee, CA), which is 30 in. in diameter, 30 in. deep, and has a 45° conical bottom (Figure 2). About 300,000 eggs were incubated in each pot. Water was introduced near the bottom at the rate of 1 gal per min and then discharged through a pipe on the side near the top of the vessel. Water flow was adjusted frequently as the eggs swelled and became more buoyant. Hatching time was 25-30 hr at 75°F. Larvae swam up and out the overflow and were collected in a basket (5.5 in. long by 9 in. wide by 5 in. deep) made of 50 mesh per in. Saran screening.

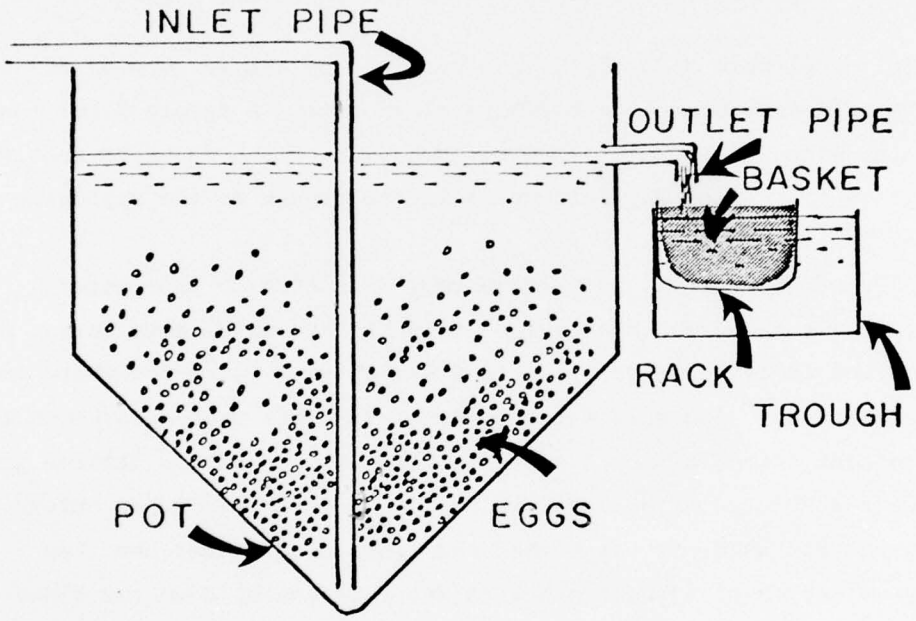

Figure 2. Hatching pot for incubation of semibuoyant eggs of white amur

12. A capability for incubation of separate groups of gynogenetic eggs was needed to test production procedures. A catfish-type hatchery was modified to achieve this objective. This hatchery was a 2- by 12-ft trough with 12 in. of water with Saran-screen baskets to hold the eggs (Figure 3). About 2500 eggs were incubated per basket and each trough held 20 baskets. The baskets (5 in. long by 8 in. wide

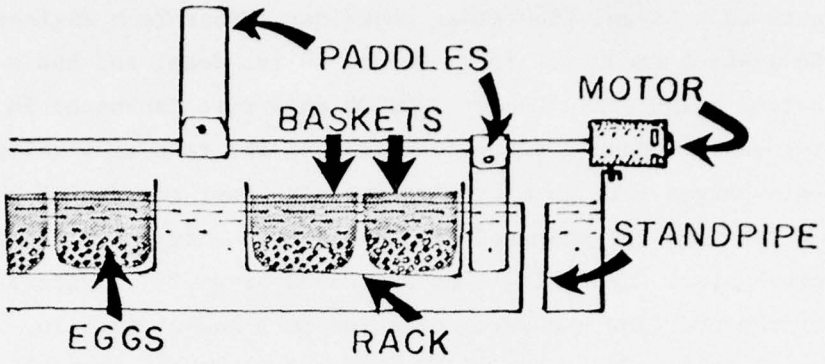

Figure 3. Basket incubator for white amur eggs

by 4 in. deep) were made of 50 mesh per in. screening. Groups of four baskets were supported by a hardware cloth rack. A paddle 9 in. long and 3 in. wide rotated at 15 rpm on each side of the racks to gently stir the eggs. Water was introduced in the trough at the approximate rate of 1 gal per min.

13. Fish eggs and larvae are very sensitive to poor water quality. A copepod which attacked newly hatched white amur larvae and fluctuation in temperature prohibited use of pond water for white amur egg incubation. Also well water to be used in the tests was known to have concentrations of ferrous iron, nitrogen, and carbon dioxide gas that were suspected of being toxic to fish eggs. Therefore, before the well water was used, it was passed through a sand filter and then through a series of three tanks with water cascading down the sides of each. In addition, two agitators were placed in the first tank.

14. Newly hatched larvae were counted by using a glass-bottomed pan illuminated from the rear. A black surface was placed 0.25 in. below the pan bottom. The 6- by 15-in. pan was marked in 1-in. grids. The larvae were removed from the baskets with a finger bowl and placed in 0.5 in. of water in the pan. After they ceased swimming, the fish were counted. The larvae were removed and then placed in a trough with Saran screening (50 mesh per in.) over the drain to prevent escape.

15. Beginning 2 days after hatching, the young were fed egg yolk.

The yolk was removed from a hard-boiled egg, frozen, and then rubbed under water between the thumb and forefinger. The resulting yolk particles were of adequate size to be eaten by the white amur fry. At 4-6 days after hatching, brine shrimp nauplii were substituted for egg yolk. Fry were stocked into newly filled 0.1-acre ponds on the seventh day and fed a complete ration of meal 5 times per week.

Gynogenesis

16. Gynogenesis was achieved in eggs "fertilized" with sperm that was irradiated to denature DNA in chromosomes. Thus, paternal influence was excluded from inheritance and progeny had exclusively maternal genetic material. Monosex broods consisting entirely of females were produced. These fish were typical white amur and should be as effective as a combination of both sexes for biological control of aquatic vegetation. By using only females the possibility of naturalization in receiving waters was minimized.

17. To produce gynogenesis, sperm from a heterologous species was used (Figure 4). Sperm from a different species assured that mistakes due to inadequate irradiation would result in hybrids that would die. In 1972, goldfish sperm was irradiated with X-rays for 50 min at 75 kiloroentgens. In 1973 and 1974 (the Israeli mirror variety) carp sperm was irradiated with UV light. A 1-mm layer of sperm solution in a 4-in.-diam Petri dish was placed on a smaller Petri dish filled with crushed ice. The milt solution was then irradiated with 6.5 milliwatt per sq in. of UV (60 min in 1973, 15 min in 1974). The shorter irradiation time was chosen after tests in 1974 showed that sperm effectiveness decreased after 60 min of UV irradiation (Figure 5). The sperm solution was mechanically agitated during UV irradiation at 50 oscillations per min.

18. In 1972, gynogenetic progeny were produced in one white amur. Of the 40,000 eggs in the spawn, 34 fish that appeared to be normal were obtained and 6 are surviving to date (June 1975). In 1973, gynogenesis again occurred in eggs from one white amur. Of 75,000 eggs 133 diploids (fish that appeared normal) were found. Resulting fry were fed hormones in an attempt to reverse the sex (see below), and 42 were stocked in

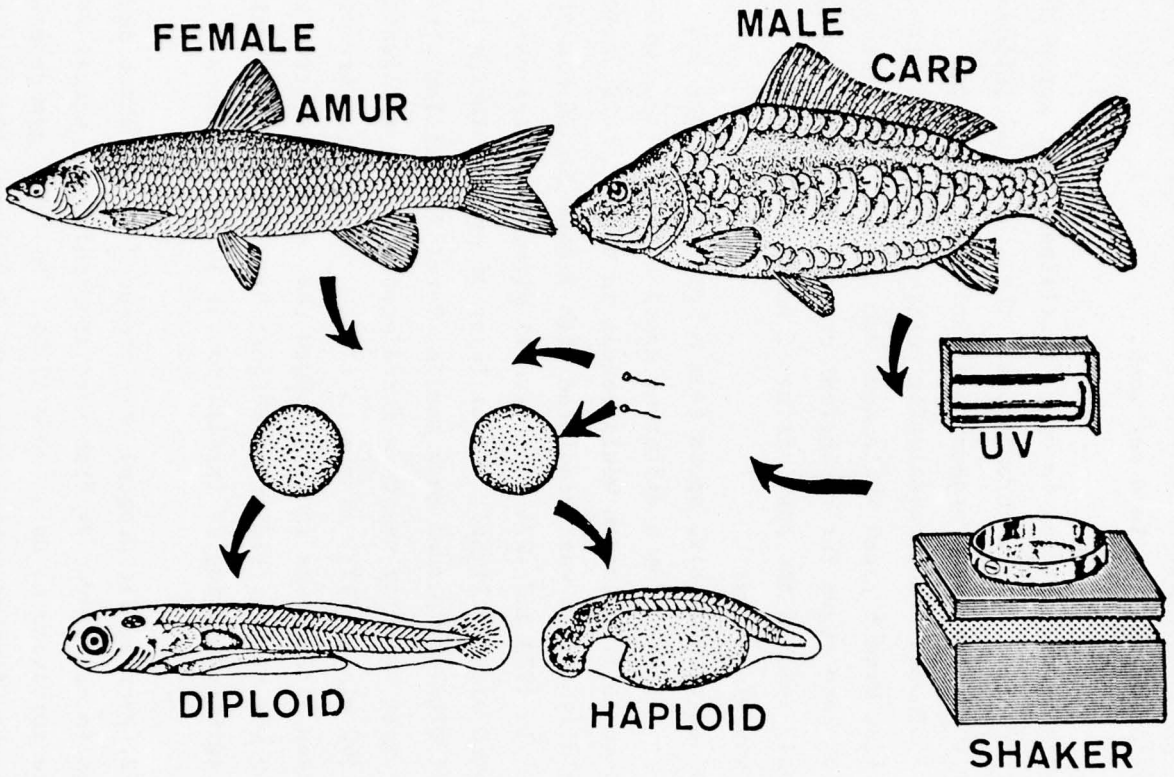

Figure 4. Gynogenesis in white amur was achieved by fertilizing amur eggs with UV-irradiated milt. Two kinds of progeny resulted: diploids that had two complete sets of chromosomes, and haploids that had one set of chromosomes. Diploids grew into typical amur and haploids died. Because male inheritance was eliminated by irradiation the offspring were exclusively female

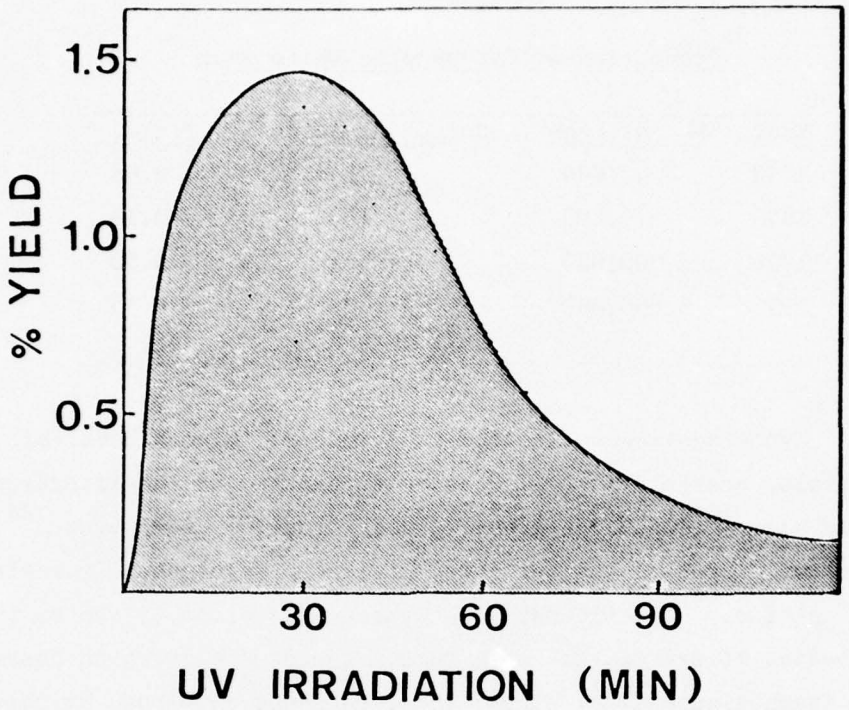

Figure 5. Effect of the duration of UV irradiation of sperm on the yield of diploid gynogenetic white amur. These data suggest that maximum yield is obtained at a UV dose which denatures DNA without altering sperm viability

five 0.25-acre ponds. In 1974, two females produced large numbers of gynogenetic progeny. Of 115,000 eggs from one fish, 4,230 amur were obtained, and of 143,000 eggs from another fish, 3,632 diploid fish resulted. Fish with lesser yields gave 805 diploids from 82,000 eggs and 1,334 from 92,000 eggs. Thus, of nearly 0.5 million eggs, 9,971 diploid gynogenetic progeny resulted. Eight other females delivered 1.4 million fertile eggs, but with no gynogenetic fish. In 1975, 5 million eggs obtained from 25 females yielded 21,100 gynogenetic fry. The production is summarized in Table 1.

19. The yield of diploid gynogenetic fish was greater in 1974 and 1975 than in the previous 2 years (Table 1). About 0.5 percent of the eggs of four fish with fertile eggs hatched. Expressed as a probability, the chances of an egg being diploid gynogenetic was 0.005.

Table 1

Production of Gynogenetic White Amur

Year	No. of Eggs	No. of Diploids	Yield, %
1972	40,000	34	0.08
1973	75,000	133	0.18
1974	2,000,000	9,971	0.50
1975	5,000,000	21,126	0.55

20. Gynogenesis was also observed in carp, bigmouth buffalo, black buffalo, and goldfish. It has been reported in the literature in striped bass,[24] loach,[25] perch,[26] brook trout,[27] brown trout,[28] rainbow trout,[29] rudd,[30] silver carp,[31] cisco,[29] sturgeon,[32] sterlet,[32] beluga,[32] plaice,[29] and flounder.[29] Gynogenesis probably can be induced in any species if care is taken to remove the dead eggs which contaminate the incubation medium. Thus, the techniques developed by this study have wide application in genetics research and selective fish breeding as well as in creating monosex populations.

21. Spontaneous gynogenesis occurs infrequently in all species. In some species cold or heat shocks increase yield of diploid gynogenetic fish. Cold temperature extremes interfere with expulsion of the second polar body needed here for diploid.[25,28] In all tests except one, cold shocks decreased the yield of gynogenetic white amur (Figures 6 and 7). Eggs from one female responded to both cold and warm shocks with increased yield (Figure 8). In loach, the response of eggs from different females to temperature varied,[25] which may explain the varied results in these experiments. Pressure and colchicine tests caused complete mortality to eggs.

22. Monosex fish were expected in gynogenesis. This is because paternal inheritance was eliminated by irradiation of sperm. This expectation was based on the assumption that sex of white amur is determined by inheritance (two X chromosomes for female; and X and Y chromosomes for male) as in carp[33] and goldfish.[20] The sex of

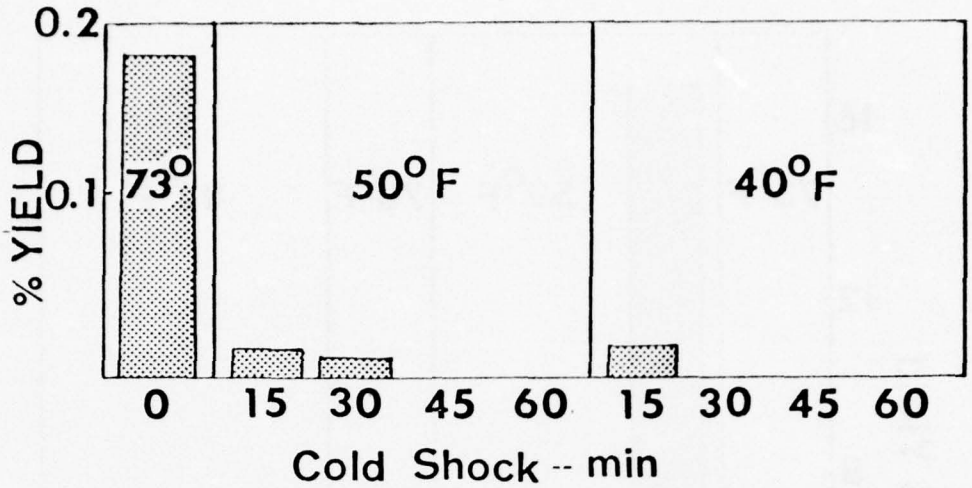

Figure 6. Exposure of white amur eggs to cold temperature beginning 1 min after fertilization did not increase the gynogenetic yield. This was contrary to expectations because other fish respond to such treatment with increased percentage of eggs that develop gynogenetically

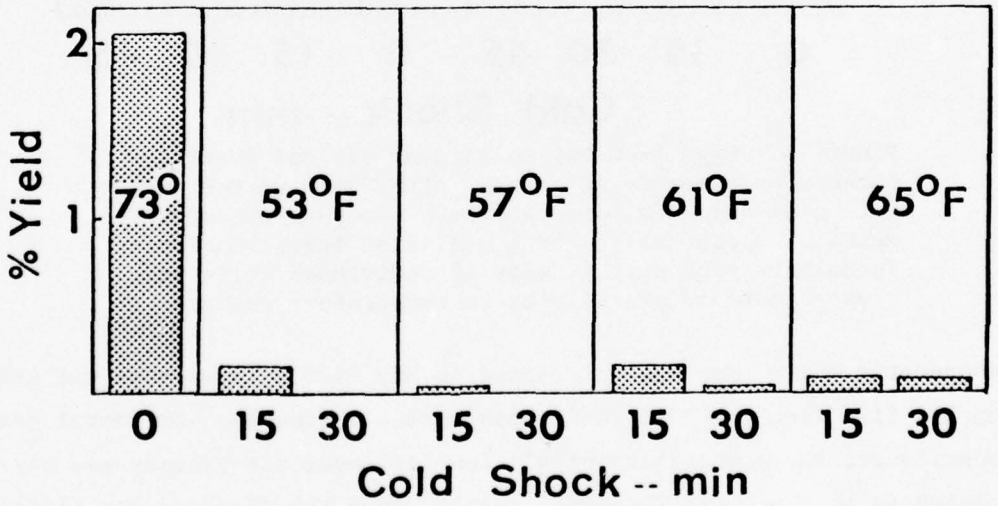

Figure 7. Mild temperature shocks were ineffective in increasing the yield of gynogenetic fish. Exposure to cool water began 1 min after fertilization

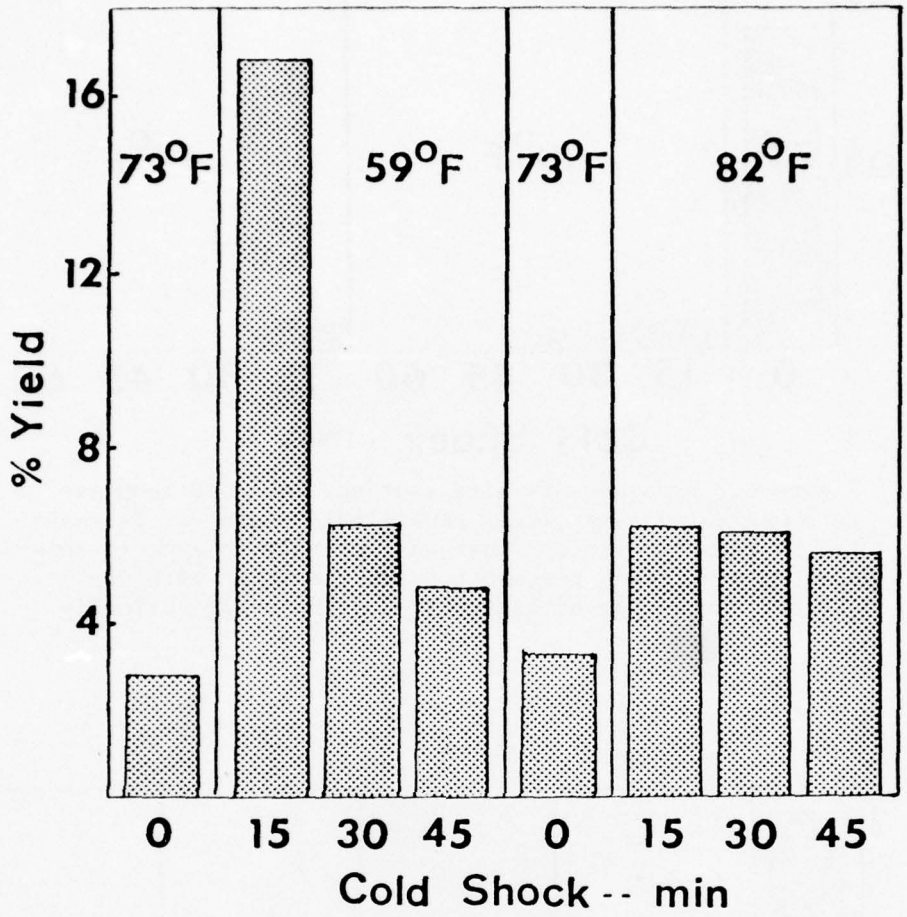

Figure 8. Eggs from one white amur yielded increased numbers of gynogenetic progeny after eggs were treated with cool and warm temperatures. Exposure to experimental temperatures began 1 min after fertilization. These data suggest that eggs of individual white amur vary in their sensitivity to temperature changes

gynogenetic white amur was determined in May 1975. Three-year-old gynogenetic fish from the 1972 year class were all females. In normal sex inheritance, the probability of all females among six progeny was calculated to be $p = 0.015$ (binomial test). Thus the observed sex ratio was not likely to be accounted for by random chance alone, which indicates that gynogenesis produces monosex fish. Gynogenetic progeny of the 1973 year class were examined and no males were found among the individuals. Males mature at 2 years of age and females at 3 years.

The absence of males suggests monosex white amur in gynogenesis.

23. Gynogenesis may not result in monosexes in every species. For example, sex inheritance in Tilapia aurea is WY chromosomes for female and YY for male.[34] Gynogenetic progeny would be of two possible types--WW females or YY males. The combination WY would not occur if the second set of chromosomes were derived from the second polar body since sister chromosomes would be recombined. The WW fish have special significance. Mating a WW female with a YY male would produce all WY female offspring.

Androgenesis

24. Androgenesis is another deviation from normal embryonic development with potential for monosex production of fish (Figure 9). Androgenesis is a rare phenomenon in which fertilization is by two sperm and the female chromosomes are lost. Reports in fish are limited to haploids produced by irradiation of eggs.[28,35,36] In these tests androgenesis was observed on three occasions in hybrid of female carp and male amur. Only six were found in the first two occasions from an unknown number of eggs. On the third occasion, 43 androgenetic amur were recovered from 2,500 fry that came from 110,000 eggs. Another pond stocked with 1,800 fry from 50,000 eggs did not yield androgenetic white amur. Androgenesis was not observed in numerous crosses of wild Cyprinus carpio and white amur.

25. Androgenetic progeny probably came from a few individual females. This probability was about 0.7, i.e., the fraction of ponds with androgenetic fish (0.5) divided by the average number of female parents involved per pond (7.5). Thus, 1 out of 14 females might be expected to give androgenetic progeny. Even in those females in which androgenesis occurred, it was not a likely event. Assuming that one female gave all 43 androgenetic progeny from 18,000 eggs, the chance of one egg developing androgenetically was 0.0002. Based on all eggs examined, the probability of androgenesis occurring in any egg from any female was 0.00003. Androgenesis is not likely to be detected unless large numbers of hybrids are examined.

26. Androgenesis is thought to result from fertilization of an

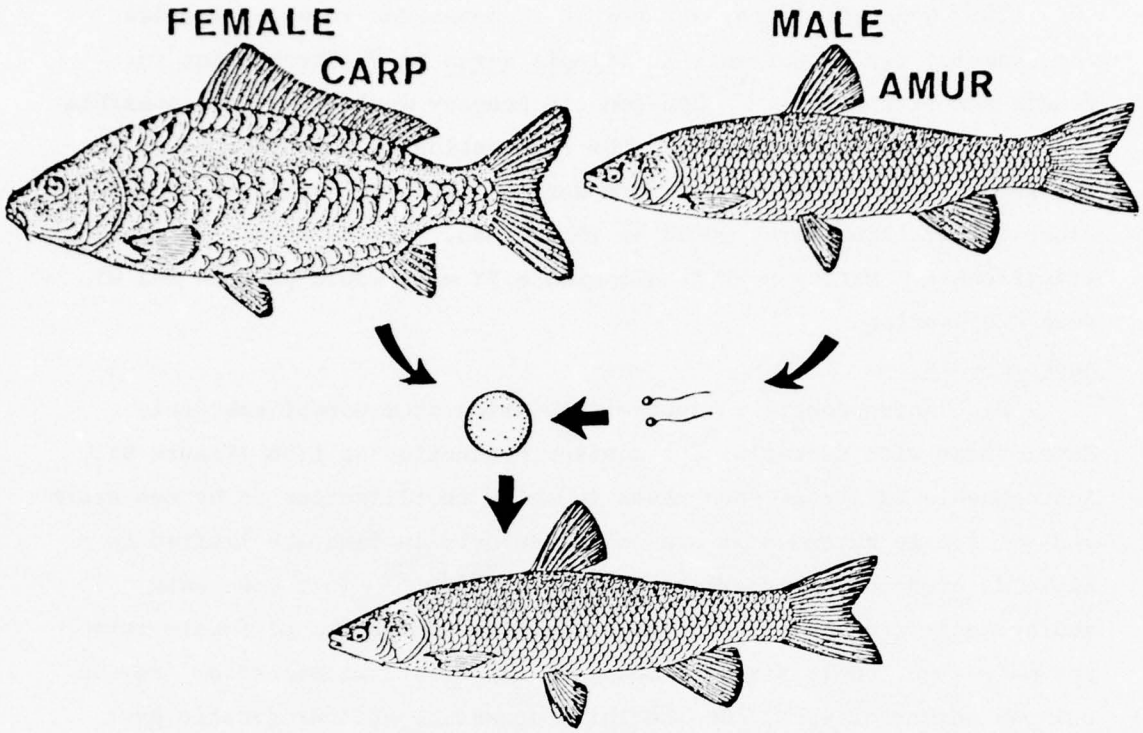

Figure 9. Androgenesis is a variation from normal embryonic development that has potential for monosex production. Fertilization is by two sperm and the female genome is lost. Offspring inherit only paternal traits from the white amur parent

egg with two sperm (Figure 9) followed by elimination of the odd set of chromosomes from the mother. Thus, the offspring contains two sets of chromosomes derived from separate sperm. There are three genotypes dependent on the sex chromosomes carried by each sperm (Figure 10). With two X-bearing sperm, a female results, an X and a Y result in a male, and YY produces an unusual male type. The YY genotype is not lethal in fish.[37,38] When bred to a normal XX female, this unusual male produces all male offspring. Thus, monosex males can be produced in the second generation. The problem is in identifying the unusual combination YY. Only one third of the androgenetic males carry this combination, and test crossing is necessary to distinguish them from the normal male type.

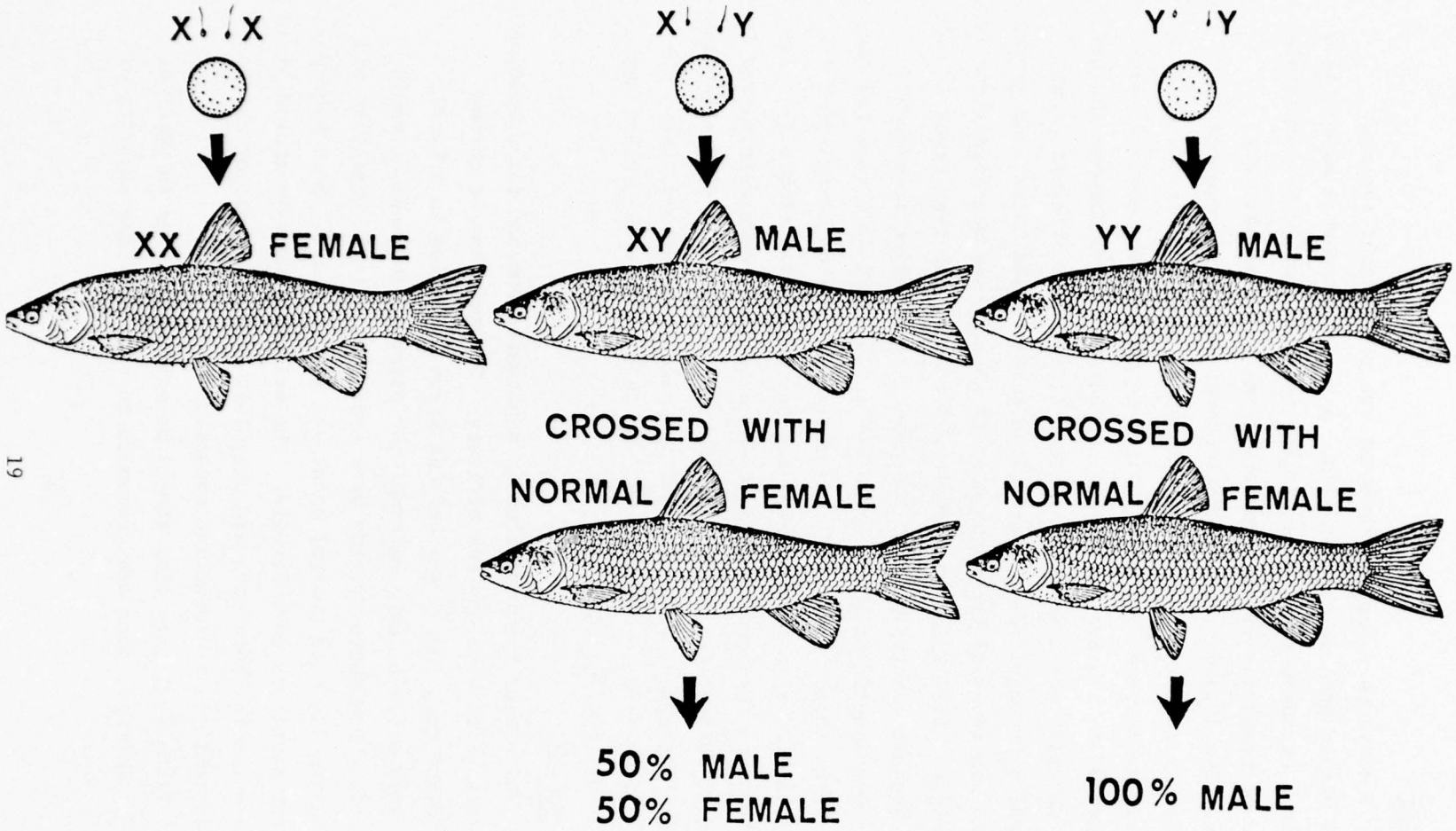

Figure 10. Androgenetic white amur have potential for production of all-male populations. Although androgenetic progeny are not monosexes, about 25 percent have an unusual combination (YY) of sex chromosomes. These YY males mated to ordinary XX females produce 100 percent male progeny

Sex reversal

27. Techniques using sex reversal are potentially the most economical way to produce monosex fish. A few sex-reversal males could sire millions of monosexed female progeny and costs would be comparable with those of producing mixed-sexes offspring by usual hatchery techniques. The disadvantage is that several years are required for rearing the sex-reversed broodfish to serve as parents. If the usual sex reversal procedures[19,20] were applied to white amur, about 5 years would be needed to achieve monosexes: 3 years to raise hormone-fed fry to sexual maturity and 2 years for a test cross. A test cross with normal females is required to distinguish sex-reversed males from normal males. The sex reversal technique used in these tests was significantly shorter (Figure 11). With gynogenetic white amur, the test cross is unnecessary because genotypic males are absent in gynogenetic progeny.

28. Gynogenetic offspring produced in 1973 and 1974 were fed the androgen, methyltestosterone [17(α)-methyl-A^4-androsten-17-(β)-01-3-one]. Hormone-fed fish totaled 1085 and controls, 1147 (Table 2). The length of time on feed and the age at which feeding began were varied in an attempt to deliver the hormone at the time of sexual differentiation. This range of treatment assuredly will produce sex-reversed fish. Effectiveness will be determined in 1975 or 1976 for the 1973 class and in 1976 or 1977 for the 1974 class.

Carp-amur hybrids

29. White amur hybridization is a potential method for producing a sterile fish with weed control efficacy. The amur has 48 chromosomes[39] and the carp 100,[40] and egg and sperm formation in a hybrid should be impossible because maternal and paternal chromosomes would not pair. In these tests hybrids were produced, but the objective of a practical sterile weed control agent was not obtained. Most hybrids died and the survivors were fertile. The evidence suggests that diploid fish die and the few survivors are polyploid (multiple sets of chromosomes) with restored reproductive capacity.

30. Probably little else should be said concerning an unsuccessful method. However, data are presented to show that the majority of

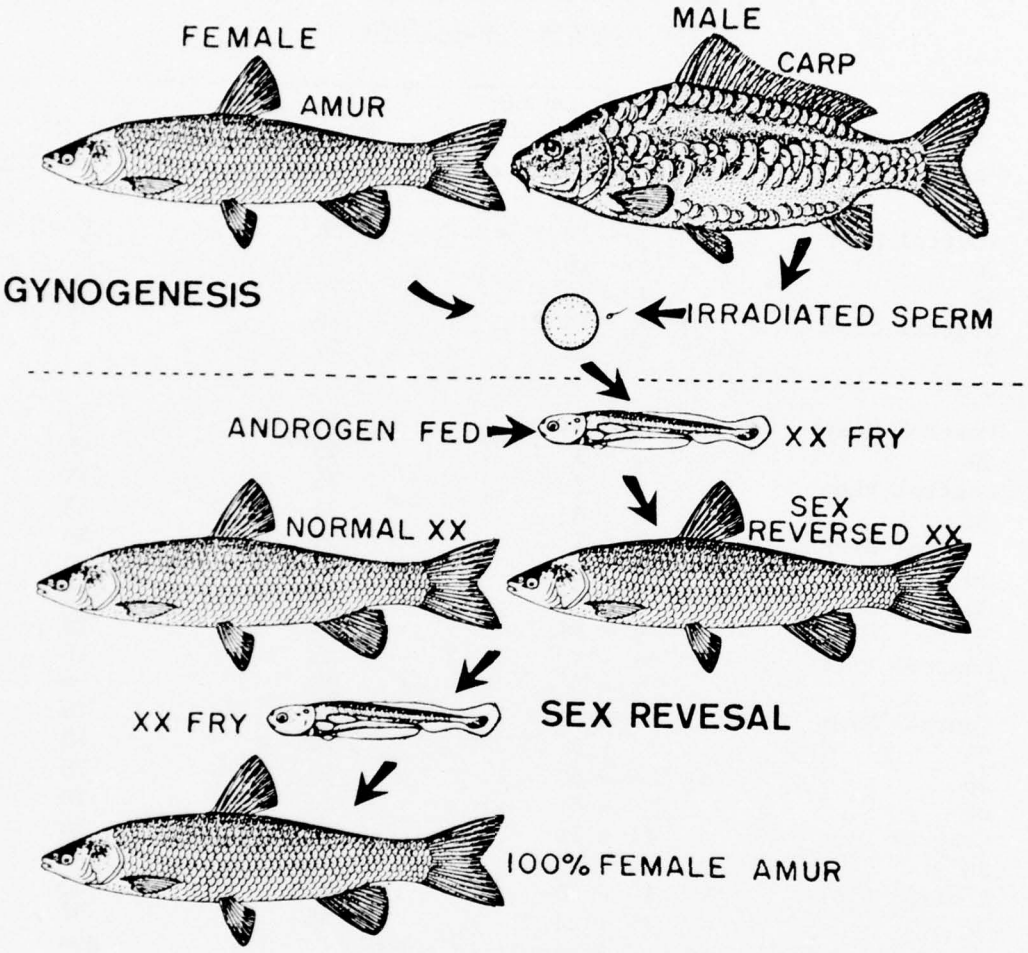

Figure 11. Sex reversal technique is the most practical method for monosex production of white amur fish. Male fish having female genotype are made by feeding androgen (male hormone) to gynogenetic young. Mating sex-reversed males (female type) with normal females in ordinary hatchery operations results in all-female broods. Costs are comparable with usual hatchery costs for producing mixed-sex broods

Table 2

White Amur (Gynogenetic) Fed Methyltestosterone* to Reverse the Sexes for Production of Monosex Populations

Treatment mg/kg Feed	Age at Beginning and End of Feeding weeks	No. of Fish Fed	No. of Fish Stocked
1973			
Control Diet	1 - 10	26	12
30	1 - 10	27	10
60	1 - 10	27	8
Control Diet	4 - 14	26	7
30	4 - 14	27	5
1974			
Control Diet	1 - 3	50	50
30	1 - 3	50	50
Control Diet	6 - 8	35	35
30	6 - 8	35	33
Control Diet	1 - 5	50	50
30	1 - 5	50	50
Control Diet	6 - 10	35	35
30	6 - 10	35	29
Control Diet	1 - 7	25	18
30	1 - 7	25	4
Control Diet	4 - 8	70	70
30	4 - 8	70	70
30	4 - 8	70	70
60	4 - 8	70	70
Control Diet	11 - 15	35	35
30	11 - 15	35	26
Control Diet	16 - 20	35	35
30	16 - 20	35	34
Control (no feed, stocked directly in ponds)			800
30	4 - 8	about 650	626
		Total Control	1147
		Total Androgen	1085

* Methyltestosterone was incorporated into the diet at either 30 or 60 mg/kg of feed. No other feed was presented during the feeding interval.

hybrids are inviable and the few survivors are capable of reproduction. The time of hybrid mortality depended on whether the carp or white amur served as the maternal parent. Hybrids of amur female X carp male invariably died during embryonic development. A few abnormal larvae hatched but never reached the active feeding stage. Makeeva and her coworkers in the Soviet Union[41,42] report similar findings; of 500,000 eggs no hybrids were produced. The reciprocal cross between carp female and amur male developed normally, although mortality was high. Of 110,000 eggs, 2,500 hatched, were fed, and stocked into a pond. Only 5 survived to 3 mo of age. In a second lot of 50,000 eggs, 1,800 were stocked but only 11 were present when the pond was drained 3 months later. Similar ratios were found when the wild carp was used as the mother. About 500 hybrid fish were produced during 3 years. These were of value because they could be used for comparison with gynogenetic and androgenetic amur (Figure 11). Carp-amur hybrids are described in References 41 and 43-45.

31. In a study of reproductive capability conducted in cooperation with Janice Hughes and Louis Richardson of the Louisiana Conservation Department, it was found that hybrids were capable of forming fertile reproductive products. Backcrosses with the carp were more successful than second generation (F_2) matings with siblings. Of 15,000 hybrid eggs backcrossed by fertilizing with carp sperm, 4,910 larvae hatched and 61 percent were abnormal. Of 1,837 larvae, 40 percent were abnormal from 17,000 F_2 eggs. Therefore, it is recommended that no further work be done on hybrids.

Purity of experimental progeny

32. Gynogenetic or androgenetic progeny may not be pure. Paternal inheritance occurs in presumed gynogenetic silver carp.[31] If gynogenetic or androgenetic amur are not pure, they might perform differently than do normal progeny. These fish look like white amur (Figure 12), but carp inheritance must be completely excluded. Morphological and biochemical evidence shows experimental progeny to be identical to normal white amur. If inheritance included carp genes, some traits would be intermediate between the two parental species.

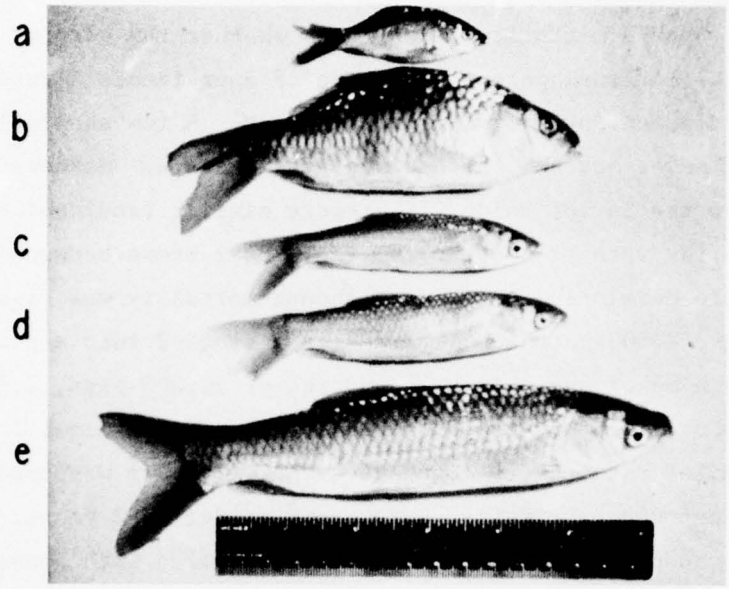

Figure 12. The white amur is morphologically distinct from carp. The carp (a) is partially scaleless. The carp-amur hybrid (b) is intermediate between carp and amur in appearance. Gynogenetic amur (c), normal amur (d), and androgenetic amur (e) are indistinguishable. This shows that gynogenetic and androgenetic amur are pure with no inheritance from the carp father

33. The number of fin rays, lateral line scales, and gill rakers distinguished white amur from carp (Table 3). Hybrids were intermediate. The dorsal and anal fins of all three forms had three (III) spinous rays, but only the carp and the hybrid had a strongly serrated third spinous ray. These characteristics in gynogenetic and androgenetic amur were similar to those of normal amur.

34. The different body dimensions were less useful in distinguishing the kinds of fish (Table 4). Only the length of the dorsal fin base clearly separated hybrids, carp, and amur; it was longest in the carp, shortest in the amur, and intermediate in the carp-amur hybrid, reflecting the number of fin rays. Head length and body depth were poor diagnostic traits. Although amur, carp, and carp-amur hybrids all significantly differed from each other, there were also significant differences in head length among normal, androgenetic, and gynogenetic

Table 3

Average Number of Fin Rays, Lateral Line Scales, and Gill Rakers in Normal, Androgenetic, and Gynogenetic White Amur, Normal and Gynogenetic Carp, and Carp-Amur Hybrids

Form	No. of Fish	Number of Fin Rays*		Lateral Line Scales*	Gill Rakers*
		Dorsal Fin	Anal Fin		
White Amur					
Normal	10	III 7.1 (0.10)	III 8.1 (0.10)	40.5 (0.53)	15.0 (0.36)
Androgenetic	10	III 7.0 (0.00)	III 8.0 (0.00)	40.6 (0.30)	14.3 (0.49)
Gynogenetic	10	III 7.0 (0.00)	III 8.0 (0.00)	40.0 (0.42)	14.7 (0.21)
Carp					
Normal	10	III 19.9 (0.31)	III 6.0 (0.00)	11.4 (1.62)	26.6 (0.29)
Gynogenetic	1	III 21.0 ----	III 6.0 ----	7.0 ----	26.0 ----
Carp X grass carp					
Hybrid	7	III 16.0 (0.62)	III 6.0 (0.00)	33.8 (1.14)	16.4 (0.20)

Note: Standard errors are in parentheses.

* A mean value enclosed by a bracket does not differ significantly from other values within that same bracket but differs significantly at the 95 percent level from all values outside that bracket.

Table 4

Average Body Dimensions of Normal, Androgenetic, and Gynogenetic White Amur, Normal and Gynogenetic Carp, and Carp-Amur Hybrids

Form	No. of Fish	Percent of Total Length*		
		Head Length	Body Depth	Base of Dorsal
White Amur				
Normal	10	21.9 (0.44)	[17.7 (0.26)	[8.1 (0.14)
Androgenetic	10	18.8 (0.30)	18.4 (0.27)]	8.3 (0.14)
Gynogenetic	10	19.2 (0.35)	19.2 (0.21)	8.2 (0.12)]
Carp				
Normal	10	[23.4 (0.45)	29.4 (0.21)	[32.4 (0.66)
Gynogenetic	1	24.2 ----	38.0 ----	34.9 ----]
Carp X grass carp				
Hybrid	7	20.9 (0.74)	31.9 (0.29)	27.8 (0.81)

Note: Standard errors are in parentheses.

* A mean value enclosed by a bracket does not differ significantly from other values within that same bracket but differs significantly at the 95 percent level from all values outside that bracket.

amur. These differences did not follow a pattern of intermediacy between species and are thought to be environmentally induced.

35. The pharyngeal teeth formula varied greatly within normal carp, making count comparisons meaningless. The carp had molar-type teeth distinctly different from the serrated, rasping teeth of the amur, and the hybrid had teeth much like the carp. Gynogenetic and androgenetic white amur were similar to normal amur.

36. Electrophoretic analysis of plasma and other body fluids is a method of detecting hybrids.[46,47] Here, the objective was to demonstrate that the gynogenetic and androgenetic white amur were not hybrids but pure white amur. Electrophoresis of hemoglobin, blood plasma proteins, esterases, and lactate dehydrogenase was done on an EC 470 and EC 490 apparatus (EC Apparatus Corp) using polyacrylamide block gel and Peacock buffer at pH 8.4. In all, 29 samples from normal white amur, 17 from normal carp, 213 from gynogenetic white amur, 17 from androgenetic white amur, 1 from gynogenetic carp, and 5 from hybrids were analyzed. The work was done by Charles J. Biggers and Don E. Schultz of Memphis State University. They found that gynogenetic fish had no paternal inheritance and that androgenetic fish had no maternal inheritance.

37. Hemoglobin from carp and white amur had three electrophoretic bands (Figure 13). The three bands in gynogenetic and androgenetic amur were identical to those in normal amur. Carp-amur hybrids had four distinct bands and one faint band not evident in Figure 12. The five bands corresponded with those in either carp or amur bands. General protein electropherograms also showed that gynogenetic and androgenetic amur were pure (Figure 14). The carp-amur hybrid more nearly resembled the carp than the amur although some general protein bands were identified as amur bands.

38. Electrophoresis of enzymes also showed purity of experimental progeny. The esterases (Figure 15) of gynogenetic, androgenetic, and normal amur had a single band, whereas carp and hybrids had three overlapping bands. Although the amur band migrated at the same rate as did the slowest carp band, the esterases of the two species probably

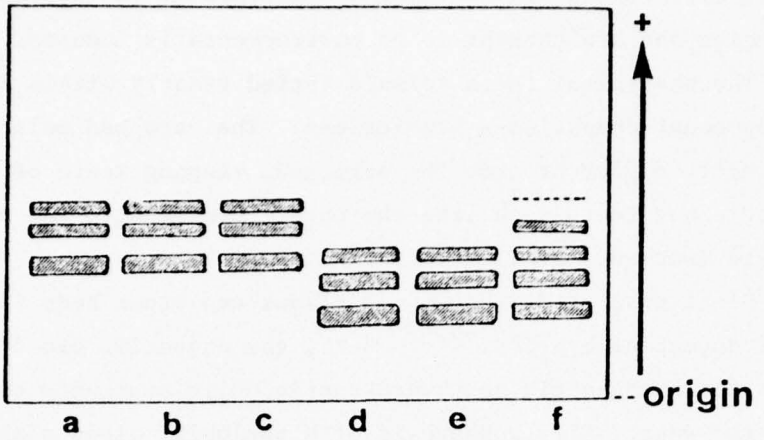

Figure 13. A tracing of hemoglobins separated by electrophoresis. Hemoglobins of different molecular structure migrated various distances depending on charge and size. These data show that amur and carp both had three hemoglobins, which were different between the two species. Hybrids had hemoglobins from both parents; whereas gynogenetic and androgenetic fish were identical to normal fish. (a) gynogenetic amur, (b) normal amur, (c) androgenetic amur, (d) normal carp, (e) gynogenetic carp, and (f) carp-amur hybrid

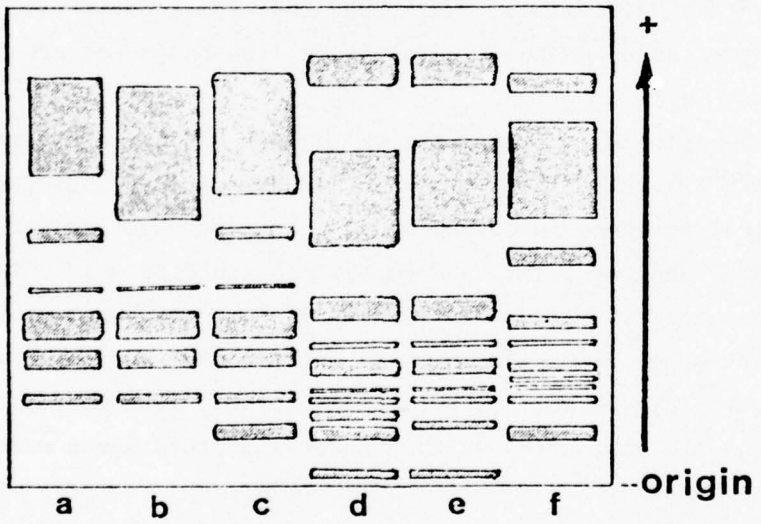

Figure 14. A tracing of the general plasma proteins separated by electrophoresis. The electropherogram shows that plasma contained many proteins. The variations between experimental fish and normal fish do not indicate inheritance from the heterologous parent. Hybrids had banding of both species and at least one band that was a hybrid molecule made from components from each parent. The legend is the same as Figure 13

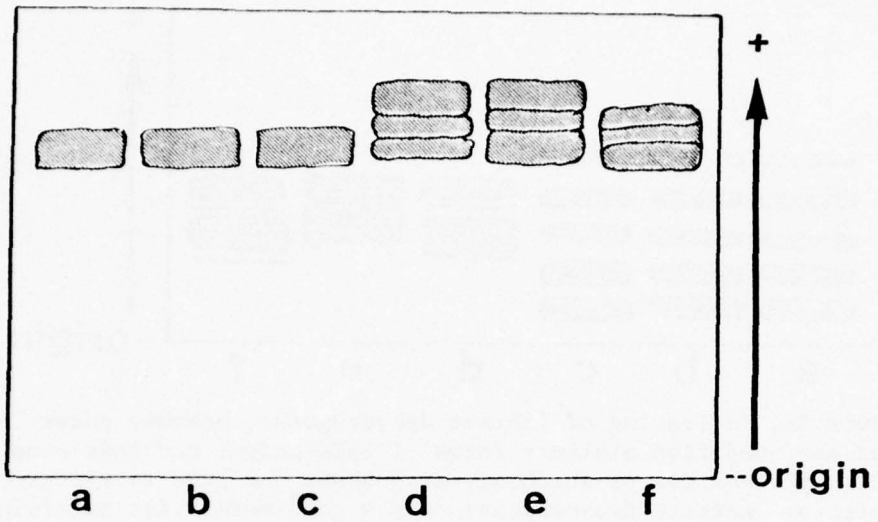

Figure 15. A tracing of esterase enzyme bands separated by electrophoresis. Amur had a single kind of esterase whereas carp had three kinds. Because carp inheritance dominated in hybrids, the absence of carp genome in gynogenetic and androgenetic amur is strongly suggested. The legend is the same as Figure 13

were molecularly different. Lactase dehydrogenase (Figure 16) in amur was composed of five bands, whereas carp and hybrids had numerous bands which migrated at the same rate. Because carp inheritance dominated in the carp-amur hybrids, the absence of the carp bands in gynogenetic and androgenetic amur strongly suggests the absence of a carp genome.

39. Comparison of chromosomes between experimental progeny was attempted. Microscopic chromosome examination was not successful, but measurement of the cell nucleus size suggested that gynogenetic, androgenetic, and normal white amur have the same chromosome number. The volume (or area as seen through a microscope) of the cell nucleus is proportionate to the number of chromosomes.[48,49] Nuclear area was the same in erythrocytes of the three groups of amur and was about 50 percent greater in the carp (Table 5). The carp-amur hybrid was expected to have a nuclear area intermediate between the two parental species, but the observed area was 50 percent greater. This suggested a tetraploid which would account for the hybrids being capable of reproduction.

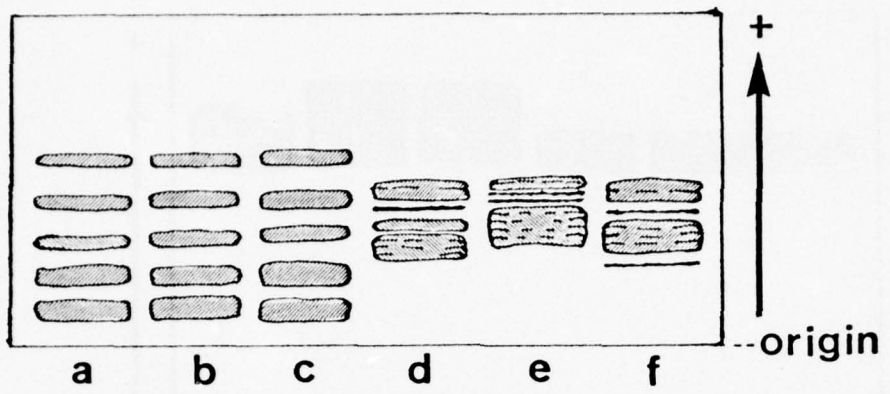

Figure 16. A tracing of lactate dehydrogenase enzymes shows that amur had five distinct forms of this enzyme and that carp had many forms all of which migrated about the same in electrophoresis. Lactate dehydrogenase was a good marker for heterologous inheritance in white amur since the presence of carp genes in the hybrid suppressed the expression of genes for the amur forms of the enzyme. The legend is the same as Figure 13

Most likely, diploid hybrids died and the relatively rare tetraploids were the sole survivors. Erythrocyte nuclear area clearly shows that both gynogenetic and androgenetic amur are diploids. This report is the first on androgenesis in which fish were diploid.

Table 5

Erythrocyte Nuclei Area of Normal, Androgenetic, and Gynogenetic White Amur, Normal and Gynogenetic Carp, and Carp-Amur Hybrids

Form	Number of Fish	Nuclear Area (um^2)*
White Amur		
Normal	14	12.4 (0.35)
Androgenetic	10	12.8 (0.29)
Gynogenetic	14	11.5 (0.29)
Carp		
Normal	14	18.3 (0.64)
Gynogenetic	1	19.8 ----
Carp X Amur		
Hybrid	10	23.5 (0.44)

Note: Standard errors are shown in parentheses.
* Values enclosed by brackets do not differ significantly from each other, but are significantly different from values outside that bracket ($P < 0.05$).

Feasibility of Producing Monosex Amur Fish for Operational Use

Scope

40. Several hundred thousand acres of water in the United States are infested with vegetation to the degree that some control would be desirable.[50] Millions of dollars are annually spent on chemicals or mechanical control programs which are usually temporary.[50] However, biological control is more economical and probably more permanent.

41. The white amur shows considerable promise for management of several kinds of aquatic plants.[8] Control of aquatic weeds can be achieved by the stocking of 20-50 fish per acre. With several hundred thousand acres, perhaps 20 million white amur could be utilized each year. It is feasible to produce this number of monosex amur. The cost of producing monosex white amur may range from a few cents to $3.00 each, depending on whether gynogenesis or sex reversal is used and whether small or larger fingerlings are needed for stocking.

Facilities

42. Monosex white amur for weed control could be produced in a two-phase development program. Through 1977 monosex fish could be produced by gynogenesis. Existing technology suggests that between 100,000 and 200,000 monosex fish could be produced each year at an average cost of about $3 each to control the vegetation in about 2,000 acres. In the second phase, beginning in 1977 or 1978, sex reversal techniques could be used to produce monosex fish for less than 5¢ each.

43. The cost of white amur delivered to treatment sites may vary, not only because of the difference between costs for the gynogenetic and the sex-reversed methods, but also because of the size of fingerling required. Small fish could be produced cheaply but they are vulnerable to predation by other fish and thus more are required. A computer model would be useful to predict the least cost method for achieving aquatic weed control using monosex fish. A first approximation is given in Table 6 for gynogenesis and in Table 7 for sex reversal techniques.

44. The models for weed control developed in Tables 6 and 7 have

as the principle variable the size of fingerling. In culture methods A, B, and C conventional hatchery ponds are used to grow the three sizes of fish. In culture method D, 3-in. fish are produced in hatchery ponds, then transferred to a nursery pond constructed adjacent to a lake with a weed problem, and after the fish reach 10 in., stocked by draining the nursery pond into the lake.

45. Several assumptions were made in calculating the models. Land needed was 125 percent of the pond surface area plus 40 acres.

Table 6

Cost of Producing and Distributing Monosex (Gynogenetic) White Amur of Different Sizes*

	A	B	C	D
Size of Fingerlings, in.	3	5	10	10 (in nursery pond)
Fingerlings Needed	200,000	150,000	100,000	100,000
Capital costs (in thousands of dollars)				
Hatchery building	500	500	500	500
Wells at $10,000 each	20	20	20	20
Land at $1,000/acre	68	62	120	93
Pond construction	32	27	24	26
Major equipment	38	36	42	30
Totals for capital	658	645	706	669
Average (5 yr)	132	129	141	134
Annual costs (in thousands of dollars)				
Salaries	136	125	134	134
Fixed costs	28	26	28	27
Distribution costs	2	3	10	1
Totals for annual	166	154	172	162
Avg. capital and annual	298	283	313	296
Treatment cost/acre	$149	$142	$156	$148

* A program of this size would control weeds in about 2,000 acres and would be feasible in 1975 to 1977.

Pond construction costs were $1500 for 1 acre, $6000 for 5 acres, $10,000 for 20 acres, and $50,000 for a 100-acre nursery pond. The major equipment included tractors, fish hauling trucks, and feeding equipment. Staffing included four supervisory and technical personnel, one secretary, two maintenance persons, and a varying number of semi-technical and labor personnel, depending on the quantity of fish being raised. Fixed costs were set at 17 percent of the other annual costs.

Table 7

Cost of Producing and Distributing Monosex White Amur (Produced by the Sex Reversal Technique) of Various Sizes*

	A	B	C	D
Size of Fingerlings, in.	3	5	10	10 (in nursery pond)
Fingerlings Needed, million	40	30	20	20
Capital costs (in thousands of dollars)				
Hatchery building	500	500	500	500
Wells at $10,000 each	60	120	900	230
Land at $1,000/acre	555	650	5040	4290
Pond construction	305	313	2009	1129
Major equipment	96	121	600	80
Totals for capital	1516	1704	9049	6229
Average (5 yr)	303	341	1810	1246
Annual costs (in thousands of dollars)				
Salaries	212	332	1300	204
Fixed costs	51	86	330	47
Distribution	20	60	200	11
Totals for annual	283	478	1830	262
Avg. capital and annual	586	819	3640	1508
Treatment cost/acre	$1.46	$2.05	$9.10	$3.70

* Weed control could be achieved on about 400,000 acres and would be feasible by 1977 or 1978.

Distribution costs did not include salaries or truck purchase and were set at $100 per trip for hauling 3000 lb of fish 200 miles. Overhead costs were not included. Treatment costs per acre were based on annual costs plus the capital costs prorated on a 5-year project. Relative survival was assumed to be 50 percent for 3-in., 75 percent for 5-in., and 100 percent for 10-in. fingerlings. Not included are developmental costs ($105,000 to 1975) and costs of monitoring and gathering background data.

46. The objective of identifying the most practical procedures for producing a weed control agent was realized. For gynogenesis (Table 6) there did not appear to be much difference in costs between the four procedures. Even though the cost of $156 per acre was higher than the other costs, model C was chosen as the most practical because weed control would be more predictable than it would when smaller fish were used. For the sex reversal technique (Table 7), the cost per acre of weed control was much less than with gynogenesis and there were considerable differences among the 4 models. The 3-in. fish gave control at a cost one-sixth that for the 10-in. fish. It was still relatively cheap to produce 5-in. fish, and these would be less vulnerable to predation, thus giving more consistent control. Method B is recommended for use in the sex reversal technique. Although these models obviously are tentative, they do allow some planning of strategy to achieve aquatic weed control at the least cost.

Cost-benefit

47. The cost of weed control using monosex amur produced by sex reversal is much less than that for chemical or mechanical removal. The chief advantage of biological control is that effects are more permanent than with other methods. Production of monosex fish should be only slightly more expensive than that of normal bisexual populations of male and female fish.

48. The benefits of weed control are many. Navigation on inland waters, water movement in canals,[50] and recreational activities such as fishing[51] and water skiing are all enhanced by weed control. The advantages of white amur over chemical control are costs and the gradual

removal of vegetation, thereby avoiding oxygen depletion and bursts of nutrients that often cause blooms of algae. Amur use results in gradual release of nutrients tied up in plants,[52] thus permitting the nutrients to be incorporated into organisms in food chains leading to desirable species of fish.[53] There may be some adverse effects on waterfowl or other wildlife that depend on aquatic vegetation for food,[14,54] but use of monosex white amur is justified because risks associated with the release of this exotic species are minimized with little change in the cost or benefits. Monosex fish would be acceptable because any damage would be temporary, and control of population size would minimize damage that does occur.

Operational problems

49. Several factors might affect the efficacy of white amur as a biological control agent. The most important uncertainties are:

a. Possible "inbreeding" in gynogenesis

b. Size-related predation by bass

c. Emigration

d. Effective life span

50. Because gynogenetic inheritance is entirely maternal, the genetic constitution of offspring is similar to offspring produced by sexual self-fertilization. Thus, gynogenetic progeny are highly homozygous[28] (genetically homogeneous), which in plants and animals generally results in reduced vigor. Growth and survival may diminish, and gynogenetic white amur may not be as effective in controlling aquatic plants as is the bisexual variety. However, females grow more rapidly than males[55] and probably eat more vegetation. This might offset the decreased vigor. It is speculated that if vigor deprivation occurs in gynogenesis, then the sex reversal technique is likely to give monosexes with hybrid vigor, and these should perform better than the average normally produced fish. Because gynogenesis is an interim method, the problem of reduced vigor is not a serious consideration.

51. Predation by bass and other carnivorous fishes potentially is a serious problem for economical use of white amur. White amur smaller than 8 in. are readily eaten by bass. In waters with dense

vegetation, escape may be possible and smaller fish might be stocked. In making models of production costs (Tables 6 and 7) it was assumed that 50 percent of the 3-in. fish and 25 percent of the 5-in. fish would be lost. In fact, there are no data to support such figures. Of urgent need is information on predation losses of newly stocked white amur by established fish populations.

52. Emigration of white amur from the stocking site is likely, especially as fish reach sexual maturity. The fish is adapted for river life and migrates long distances, both in its native habitat[55] and in the United States.[54] Migration is not limited to fresh water. In the Soviet Union white amur gained access to the Ural River by crossing the Caspian Sea (brackish water) from the Volga River.[56] Emigration has two major impacts. First, effective management of aquatic vegetation is impossible if the fish do not remain in the release area. Second, fish may congregate in waters where they are not needed and cause environmental damage. Research on fish movement is needed.

53. Of less pressing need is an estimate of the performance span of white amur for effective weed control. White amur live at least 10 years, but their capacity for vegetation consumption becomes progressively less as the fish grow older. It was observed that Najas weed reappeared in ponds after 20 amur per acre had reached a size of about 30 lb. David L. Sutton* found similar results in Florida. Weed control for 6 years is estimated,[4] but regardless of the effective period, the use of amur is more economical than are other methods of weed control.

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* Personal communication.

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APPENDIX A: BIBLIOGRAPHY ON WHITE AMUR

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