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SPAWNING THE GRASS CARP FEMALE X BIGHEAD CARP MALE

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ABSTRACT

Methods and procedures for artificially spawning the female grass carp *(Ctenopharyngodon idella)* X male bighead carp *(Aristichthys nobilis)* are presented. Broodstock selection and treatment, hormone injections, ovulation, fertilization, hatching techniques and stocking rates are discussed. This paper outlines the procedures necessary to successfully produce hybrid grass carp.

INTRODUCTION

Several fish species have been tested to control aquatic vegetation (Kilgen and Smitherman, 1971), but the grass carp *(Ctenopharyngodon idella)* has been reported to be one of the most promising (Swingle, 1957). Grass carp were introduced into the United States in 1963 by personnel at the U. S. Fish and Wildlife Service's Fish Farming Experimental Station, Stuttgart, Arkansas and at Auburn University, Auburn, Alabama (Stevenson, 1965; Guillory and Gasaway, 1978). Although two decades have passed since this introduction (Baily, 1974), the grass carp remains highly controversial because some biologists fear that this fish may reproduce and become established in natural waters (Kilgen and Smitherman, 1971; Forester and Lawrence, 1978). Restrictions on the importation of grass carp in many states (Cassani, 1981) created a need for an alternative species for nuisance aquatic vegetation control that would allay most fears associated with the grass carp, primarily reproduction and environmental degradation. Therefore, in May 1979, the Arkansas Game and Fish Commission produced the F_1 hybrid of female grass carp and male bighead carp *(Aristichthys nobilis)*. The Fish Farming Experiment Station working in conjunction with J. M. Malone, a commercial fish farmer, produced the hybrid during the same season. Marian and Krasznai (1978) had reported the successful production of this intergeneric hybrid in Hungary and their work stimulated the initial interest in this hybrid in the U. S.

Although the total progeny of the 1979 year class cross were initially reported to be triploid and expected to be sterile (Beck et al. 1980), subsequent investigations (Drs. Beck and Biggers pers. comm.) revealed that some hybrids in the 1980 and 1981 year classes were diploids. It has not been confirmed that the 2N (diploid) hybrid is sterile although Tom Jackson, Columbia National Fisheries Center, U. S. Fish and Wildlife Service, Denver, speculates that the diploid hybrid is sterile based on other available literature (pers. comm.). The percentage of diploids present varies and altering production techniques may increase the triploid to diploid ratio (Mr. J. M. Malone, pers. comm.). Acceptability of the hybrid as a substitute for grass carp will be directly linked to the question of sterility, since most fears associated with grass carp concern its reproductive potential (Bailey, 1972). Many researchers are investigating the efficacy of the hybrid grass carp for aquatic vegetation control as evidenced by a special session on the "Hybrid Grass Carp: Biology, Management, and Potential for Aquatic Plant Control" conducted at the 11th Annual Meeting of the American Fisheries Society, 1981.

At the Joe Hogan State Fish Hatchery in Lonoke, numerous inquiries have been received from federal, state, and private facilities concerning specific procedures for production of the hybrid grass carp. A report on spawning and rearing of grass carp in Arkansas (Bailey and Boyd, 1970) describing initial attempts at spawning the grass carp and a publication containing information on spawning the bighead carp (Henderson, 1979) have been the only sources of available information that could be furnished to interested persons. Procedures outlined in these publications must be combined and modified to be used successfully. Information concerning the spawning of the hybrid grass carp has not been previously published. This paper consolidates information from publications by Bailey and Boyd (1970) and Henderson (1979) and incorporates several refined procedures for the production of hybrid grass carp.

BROODSTOCK SELECTION AND TREATMENT

The selection of gravid, mature, healthy broodstock cannot be over emphasized. Most unsuccessful attempts at producing hybrid grass carp can be attributed directly to fish that were immature or in poor condition.

Broodfish can be maintained throughout the year at the rate of 250 individuals per ha or at 25 individuals per ha in polyculture with catfish broodstock. Grass carp or grass carp and catfish in polyculture should be fed daily a manufactured extruded (floating) catfish feed at about 3.0% of their body weight or 35 kg per ha, whichever is less. Feeding should be discontinued when dissolved oxygen levels in the pond are less than 3 ppm. During the winter months, feeding rates should be reduced to 2.0% of body weight or 10 kg per ha, whichever is less.

Many bighead carp males are sexually mature at year three, but grass carp females typically do not mature sexually until year four. Sexually active bighead carp males exhibit pearl organs on the dorsal side of the pectoral fins: these fins are rough and abrasive in the spring. Male bighead carp can usually be manipulated in the spring (even before a hormone injection) to release a small amount of milt. On the basis of these two characteristics it is easy to separate sexually active male bighead carp from females and sexually inactive males.

Sexually mature female grass carp exhibit distended abdomens that are flaccid as a result of egg content. For fry production purposes, grass carp females heavier than 5 kg are preferred over smaller females. In Arkansas, broodstock selection and spawning is usually performed in early May. Brood ponds are seined and fish carried in wet burlap bags to tanks containing oxygenated pond water for transport to the spawning building. Generally, the male bighead carp and female grass carp are held in separate tanks during the hormone injection phase. All brood fish should be held in water at temperatures near 23 C during the hormone injection series. Sufficient agitation, aeration, and water exchange must be provided to maintain dissolved oxygen levels above 5 ppm. Holding tanks must be covered to prevent broodfish from jumping out of the tanks.

Broodstock may be treated daily with a prophylactic continuous bath treatment of 500 ppm furacin or 1 ppm acriflavin before hormone injections are administered. After the injection series has been initiated, all treatments should be discontinued since the fish should be disturbed as little as possible. Generally, broodfish should be kept in holding facilities for only a few hours before starting the injection series since the fish may become quickly damaged, especially in concrete tanks.

HORMONE INJECTIONS

The Arkansas Game and Fish Commission has evaluated several hormone dosage levels and injection schedules (Bailey and Boyd, 1971; Henderson, 1979) and found the procedure presented in Table 1 to be the most predictable even though others have been used satisfactorily. Fish are captured head first and restrained in burlap sacks during the injection period. The human chorionic gonadotropin preparation is administered intramuscularly with a 22-guage needle into the dorsal musculature. The dry carp pituitary preparation is administered intraperitoneally with an 18-gauge needle through the base of a pelvic fin. The larger needle is necessary for the pituitary preparation, since the acetone dried pituitary tissue is suspended in sterile water and does not dissolve. Any large clumps of pituitary tissue that will not go through the needle may be discarded. No more than 2 cm^3 of liquid per injection should be given to a fish.

Although male bighead carp receive only a single injection of either hcg or dry carp pituitary, female grass carp receive a series of three injections at intervals of 24 h. It is advisable to vary the last injection given to the female grass carp so that no more than two or three fish are scheduled to ovulate at one time. The time adjustment of this last injection (+ 6 h from the stipulated 24 h interval) does not appear to adversely affect ovulation success. If male bighead carp are limited, they may be reinjected with 220 IU/kg of hcg each day for reuse the next day.

Table 1. Hormone Injection Schedule for Grass Carp Females and Bighead Carp Males.

Species and Sex	Age	Time After First Female Injection: 0-h	24-h	36-h	Approximate Time Interval From Last Injection Till Spawning
		First Injection	Second Injection	Third Injection	
Grass Carp Female	4 years or older	HCG* 220 IU/kg (100 IU/lb)	HCG 1,870 IU/kg (850 IU/lb)	Dry Carp Pituitary** 2.2 mg/kg (1 mg/lb)	10-12 h
			First Injection		
Bighead Carp Male	3 years or older	- - -	HCG 100 IU/kg (45 IU/lb) or Dry Carp Pituitary 0.22 mg/kg (0.1 mg/lb)	- - -	34-36 h

* HCG is the abbreviation for Human Chorionic Gonadotropin which is administered intramuscularly.
** Dry Carp Pituitary is dissolved in sterile water and is administered intraperitoneally.

OVULATION AND FERTILIZATION

Female grass carp should be examined beginning at 6 h after the last (third) insection: they usually ovulate from 10 to 12 h after the last injection. However, ovulation may sometimes be delayed as much as 18 h. The abdomen of a female grass carp becomes quite flaccid when ovulation is complete, so by simply "patting" a fish on the abdomen as she swims by, one can determine whether ovulation has occurred. Fish that are near ovulation usually are sluggish and remain near the surface.

When ovulation is suspected, the fish should be placed head first in a burlap sack and turned upside down. If ovulation is complete, the fish will usually become quite rigid and begin to expel eggs in a sudden gush. The vent should then be held shut to prevent the further discharge of eggs until the female can be anesthetized and dried. The eggs should flow freely into the collecting pan when the head is held higher than the vent. Generally, at least two male fish should be used for each female fish. Males will have to be manually manipulated to induce the milt discharge into the egg pan.

We prefer the dry method of fertilization (Bonn et al., 1976); sperm and eggs are simultaneously placed in a dry container and stirred dry for about one minute before water is added. Sufficient water should be added to completely cover the eggs, and then the eggs and water mixed. During the following 10 minute period for water hardening, the water on the eggs should be decanted and fresh water added two to three times to remove blood, dead eggs and other tissues and fluids. The number of eggs can be estimated volumetrically after water hardening and, if McDonald hatching jars are used, about 100,000 eggs can be put in each jar.

HATCHING AND STOCKING

Grass carp eggs are semibuoyant and if heated water supersaturated with gases is used to hatch the eggs, it is practically impossible to keep the eggs in the hatching jars. Therefore, eggs may be allowed to overflow into aquaria where actual hatching will take place in 24 to 36 hours at water temperatures between 22 and 24 C. In some facilities, a head trough with agitation has been employed to "beat out" the excess gases and to insure that the dissolved oxygen level is near saturation. Each 38 l aquarium can accommodate 100,000 to 150,000 fry. Water inflow into the aquaria should be manipulated so that the eggs are constantly in motion and water outlets should be screened to prevent egg loss. During hatching, these screens must be cleaned periodically to prevent overflow and loss of eggs and fry.

Fry ponds should be fertilized and insect predators controlled as outlined for striped bass fry culture (Bonn et al., 1976). Fry should be stocked into prepared ponds four days after hatching at a rate of about 250,000 fry per ha. Hybrid grass carp fry do not require supplemental feeding unless natural food is limited or survival is greater than 50.0%. They will readily accept any commercial minnow meal. Hybrid grass carp survival from fry to fingerlings are in the range of 0.5% to 5.0% due to a high incidence of lethal deformities among post-larvae and juveniles.

CONCLUSIONS

By following these procedures it should be possible to satisfactorily produce grass carp female x bighead carp male hybrids. Production of hybrid grass carp by the Arkansas Game and Fish Commission has become fairly routine with a spawning success rate of about 80% for female grass carp and a return rate of 0.5 to 5% for the pond stocked fry.

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