

# THE WHITE AMUR

## A Method for Inducing Triploidy in Grass Carp and Growth Variations of Diploid and Triploid Grass Carp

by  
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### INTRODUCTION

The triploid or sterile grass carp (*Ctenopharyngodon idella* Val.) is rapidly gaining acceptance as an aquatic weed control agent in areas where diploid or fertile grass carp were once banned. In Florida, a permit system has been established by the Florida Game and Fresh Water Fish Commission enabling the use of sterile grass carp in many situations. Methods for inducing triploidy in other species (Thorgaard 1983) are expected to be similar to those necessary for producing triploidy in grass carp. However, methods presently being used by commercial producers have not been made available. This report provides a general overview of the results obtained from two studies conducted at the Lee County Hyacinth Control District during 1984 dealing with methods for inducing triploidy in grass carp and subsequent growth patterns of triploid grass carp grown together with diploid fish and grown separately. Detailed reports of both studies will be available from the senior author.

### TRIPLOID INDUCTION

Thermal shocks of fertilized eggs have been used to induce triploidy in several species of fish (Gervai et al. 1980; Hoornbeck and Burke 1981; Wolters, Libey, and Chrisman 1981). However, the timing, temperature, and duration of the thermal shock that produces the best results varies between species and to some extent among individuals. Other methods involving chemical inducers such as cytochalasin B and colchicine have also resulted in polyploid offspring (Refstie, Vassvik, and Gjedrem 1977; Allen and Stanley 1979).

Hypophysation of broodfish can be accomplished by a variety of hormone injection techniques and is reviewed by Smith and Shireman (1983). Our best results were obtained by two injections of human chorionic gonadotropin (HCG) (440 IU/kg first injection and 1870 IU/kg second injection) 24 hr apart and a third injection of carp pituitary (9.9 mg/kg) 24 hr after the second HCG injection.

Hot and cold water baths were maintained by means of a thermostatically controlled water heater-circulator or chiller-circulator. In situations where eggs were acclimated to the cold shock (temperatures preceded by ~, i.e. ~7°C), ice was used to gradually lower the temperature to the target temperature as close to 5 min after egg-sperm activation as possible. A total of 45 different combinations of shock

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temperature, timing, and duration were conducted on eggs from a total of nine females. The best combinations are listed in Table 1. The reasons for inter-female/egg variations from the same or similar inducements is unknown to us but may be related to egg ripeness, and the timing of ovulation and stripping, among other factors. The relatively low survival is disappointing, although a large female weighing 10 to 12 kg could produce upwards of one million eggs in which 5 percent survival and 30 percent triploid could still yield as many as 10 to 15 thousand triploid fry. This level was achieved on at least five occasions.

Further research dealing with egg ripeness and its relationship to thermal shock susceptibility (induced triploidy) will be required for better survival and consistency of results.

## GROWTH COMPARISONS

Growth of fry and fingerling diploid and triploid grass carp was evaluated under a variety of situations involving high and low stocking density in conjunction with various levels of food availability (duckweed, *Lemna* sp.). Diploid grass carp grew faster and had significantly ( $p < 0.05$ ) higher condition factors when grown together with triploid grass carp in every situation. When diploid and triploid grass carp were grown separately and fed to satiation with duckweed, there were no significant differences ( $p < 0.05$ ) in growth rate, condition (k), conversion, or rate of consumption. The combined effect of a slightly more efficient feed conversion rate and higher consumption for diploid fish is probably responsible for their faster growth when grown together with triploid fish competing for the same resources. The physiological and or behavioral reasons for this phenomenon are unknown.

The differences between the rate of growth between diploid and triploid grass carp can be utilized to presegregate diploid from triploid fish, especially at the fry and fingerling stage, so as to reduce the time spent certifying ploidy by means of a

Table 1  
Thermal Shock Treatments Resulting in the Highest Percentage  
of Survivors and Triploid Offspring

Treatment °C	Ambient Temp., °C	Minutes After Activation	Duration	Percent Viable*	Percent Triploid**	Date†	Percent Viable* Control
5	23.8	4:45	6:00	78	18	15 Apr	82
5	23.0	4:30	30:00	2	67	24 May	99
5	23.0	4:00	25:00	16	33	02 Jun	87
5	23.0	4:00	27:00	3	100††	02 Jun	87
~7‡	23.0	2:00	25:00	12	67	20 Jun	53
~7	23.0	2:00	27:00	4	33	20 Jun	53
~7	23.0	2:00	30:00	7	83	20 Jun	53
~7	23.0	2:00	25:00	13	50	25 Jun	94

\* Survival at 6 to 8 hr after egg-sperm activation (blastula stage).

\*\* Determined from chromosome preparations for 12 larvae from each treatment.

† Different dates indicate results from different females.

†† Eggs from second stripping, 51 min after first stripping.

‡ Acclimated to the target temperature  $\pm 1^\circ\text{C}$  before 5 min after egg-sperm activation.

Coulter Counter. Visual segregation of triploid fish which are less plump can be accomplished with larger fish ( $\geq 300$  mm total length) with accuracy exceeding 95 percent if the fish have not been overcrowded or underfed. This is more time-consuming since each individual must be examined and the accuracy may vary between individuals making the inspection.

## REFERENCES

- Allen, S. K., Jr., and Stanley, J. G. 1979. "Polyploid Mosaics Induced by Cytochalasin B in Landlocked Atlantic Salmon *Salmo salar*," *Transactions of the American Fishery Society*, Vol 108, pp 462-466.
- Gervai, J., Peter, S., Nagy, A., Horvath, L., and Csanyi, V. 1980. "Induced Triploidy in Carp, *Cyprinus carpio* L.," *Journal of Fish Biology*, Vol 17, pp 667-671.
- Hoornbeck, F. K., and Burke, P. M. 1981. "Induced Chromosome Number Variation in the Winter Flounder," *Journal of Heredity*, Vol 72, pp 189-192.
- Refstie, T., Vassvik, V., and Gjedrem, T. 1977. "Induction of Polyploidy in Salmonids by Cytochalasin B," *Aquaculture*, Vol 10, pp 65-74.
- Smith, C. R., and Shireman, J. V. 1983. "White Amur Bibliography," Miscellaneous Paper A-83-7, prepared by the University of Florida, Gainesville, Fla., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Thorgaard, G. H. 1983. "Chromosome Set Manipulation and Sex Control in Fish," *Fish Physiology*, Vol IX B, Hoar, W. S., Randall, D. J., and Donaldson, E. M., eds., Academic Press, New York, pp 405-434.
- Wolters, W. R., Libey, G. S., and Chrisman, C. L. 1981. "Induction of Triploidy in Channel Catfish," *Transactions of the American Fishery Society*, Vol 110, pp 310-312.