Triploidy Induction in Largemouth Bass, *Micropterus salmoides*

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ABSTRACT. Triploid largemouth bass, *Micropterus salmoides*, were produced by using hydrostatic pressure treatments five minutes after fertilization. Treatments ranged from 4,000 p.s.i. for 3 minutes to 8,300 p.s.i. for 1 minute. All treatments produced triploids. A treatment of 8,000 p.s.i. for 1 minute was best, yielding 100% triploids and a relatively low mortality.

INTRODUCTION

Increasing the number of chromosome sets in organisms has proven beneficial in several agricultural applications, particularly in situations where individuals with extra sets of chromosomes appear to exhibit increased growth potential. Chromosome set manipulation has been attempted in several species of fishes, with varying degrees of success (Thorgaard 1983).

Most of the work with fishes has involved the induction of triploidy. The potential benefits of triploids to aquaculture and/or fishery management are numerous. In many studies (Purdom 1972, 1976; Valenti 1975; Wolters et al. 1982; Chrisman et al. 1983; Ueno et al. 1986), triploids have been shown to grow faster and

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attain larger ultimate size than diploid siblings. This may be due to the fact that triploids are typically sterile (Purdom 1976; Cassani and Caton 1986) and the energy normally used for reproduction is invested in somatic growth (Thorgaard and Allen 1987). The elimination of reproduction may be useful for the introduction of non-indigenous fishes or where the potential for stresses from overpopulation exists. Additionally, the stocking of triploid (sterile) fish is a safer method for introducing novel strains that have been selected or bred for specific traits, because triploids will minimize impacts on natural gene pools.

Triploids can be produced by causing a fertilized egg to retain its second polar body nucleus (Thorgaard 1983). Current methods used to induce triploidy include chemicals, hydrostatic pressure, and thermal shocks subsequent to sperm penetration of the egg (Streisinger et al. 1981; Thorgaard 1983; Benfey and Sutterlin 1984; Curtis et al. 1987; Thorgaard and Allen 1987). Near-lethal temperature or pressure appears to yield the highest incidence of triploid individuals (Thorgaard 1983); hydrostatic pressure treatment appears to be the more efficient technique (Cassani and Caton 1986).

The largemouth bass, *Micropterus salmoides*, is an important sport fish throughout most of North America, receiving both state and federal fishery management attention. The benefits usually associated with triploidy offer a potential for the species to expand its size capability. This potential, coupled with restrictive size limits, may allow fisheries managers to produce trophy-sized largemouth bass in some locations. It would be most useful in small impoundments or similar situations where reproductive limitations are needed or supplemental stocking is feasible. Triploidy induction of largemouth bass to our knowledge has never been reported. The objective of this paper is to describe a procedure using hydrostatic pressure shocks to induce triploidy in largemouth bass.

METHODS AND MATERIALS

Largemouth bass used in the experiments were obtained from the wild and maintained at the Texas Parks and Wildlife Department's Heart of the Hills Research Station (HOH). Gametes were obtained by stimulating reproductive condition in adult fish through photoperiod and temperature manipulation, then further inducing ovula-

tion and sperm production by injecting fish with human chorionic gonadotropin at 4,000 I.U./kg (Stevens 1970). Ripe eggs were stripped from females into 1.8-l dishes. Milt was stripped from males, collected by pipette, and mixed with the eggs. Eggs were fertilized in enough ambient water (approximately 22°C) to just cover them.

Gametes obtained from two females and two males were mixed and apportioned evenly among treatments. Eggs were then subjected to six different pressure treatments, 5 minutes after fertilization.

In each hydrostatic pressure treatment, 100-500 eggs were gently poured into a mesh basket and subjected to high pressure in a pressure chamber. The pressure chamber was similar to that described in Curtis et al. (1987).

Pressure treatments ranged from 4,000 p.s.i. for 3 minutes to 8,300 p.s.i. for 1 minute (Table 1). After treatment, eggs were removed to aquaria for hatching. All pressure treatments occurred under ambient temperature conditions (21-25°C). Controls were groups of fertilized eggs obtained in the same way, at the same time, and from the same adult fish as above, but not subjected to pressure.

In control and experimental aquaria, dead eggs were removed daily and counted. Aquaria were cleaned daily, and 50% of the water in each aquarium was replaced weekly with fresh water. Upon hatching, which occurred after approximately 60 hours, all larvae were fed live brine shrimp, *Artemia gracilis*, twice daily to satiation.

Ploidy levels from a subset of control and experimental individuals were assayed using flow cytometric analysis following the procedures of Gold et al. (in press). Briefly, approximately 5 mm^3 of tissue (internal organs and/or muscle) were taken from 1- or 2-month-old fish (15-20 mm standard length) and placed in a cryovial containing 60-80 μl of storage buffer. The storage buffer contained 85.5 g sucrose (250 mM), 11.76 g citric acid-trisodium dihydrate (40 mM), and 50 ml dimethylsulfoxide (DMSO) in 1 liter of distilled water at pH 7.60. The cryovials were then immersed in liquid nitrogen, transported for approximately 4 hours (to College Station, TX) and maintained at −80°C until they were analyzed.

Samples were prepared for flow cytometry by thawing the material at room temperature, douncing the tissue firmly in a 2-ml Kontes

dounce with 0.6 ml of 75% sodium citrate, and filtering the resultant solution through a 41 μ nylon filter. The filtered solution was centrifuged for 3-8 seconds in a microcentrifuge and the pellet resuspended in 0.3-0.5 ml of staining solution. The staining solution contained 2 g sodium chloride, 0.14 g PIPES buffer, 0.07 g disodium EDTA, 10 μg of DNAse-free (boiled for 5 min) RNAse, and 12.5 mg propidium iodide in 200 ml of distilled water at pH 7.50. Centrifugation and resuspension were repeated twice. The prepared sample was kept on ice or in a refrigerator at 4°C for 20 minutes to ensure staining of nuclei. Erythrocytes from chicken, *Gallus domesticus*, and koi variety of common carp, *Cyprinus carpio*, were used as internal standards and were prepared as described above. An Ortho Cytofluorograph 50H[1] equipped with an 8W Lexel Model 95 ion laser, a flow cell assay system, and an Ortho 2140 Data Acquisition System was used to determine ploidy levels of individual fish. The laser was set at a wavelength of 514 nm with 1.8-W output. Details of the use of signals for selecting and recording histogram data are in Gold et al. (in press).

RESULTS AND DISCUSSION

Hydrostatic pressure treatments induced triploidy in largemouth bass. DNA content in triploid largemouth bass was 50% greater than that of diploid bass, as determined by flow cytometry. Diploid specimens had about 2 picograms of DNA per nucleus, while triploids had approximately 3 picograms of DNA per nucleus (Table 1).

Pre-hatching mortality in the treatment groups was closely approximated by mortality in respective control groups (Figure 1) and was, therefore, apparently due to factors other than the treatments. Thus, hydrostatic pressure treatments at these levels did not appear to increase mortality. Mean mortality of treated fish and controls was 53% and 50%, respectively. We chose pre-hatching mortality for comparison because previous experience showed most handling-induced damage and developmental problems are manifested during this period.

1. Use of trade names does not imply endorsement.

TABLE 1. Ploidy of hydrostatically-shocked largemouth bass assayed by flow cytometric analysis of erythrocyte nuclei. Control groups from each of the pressure treatments are combined. DNA content is in picograms (pg). Coefficient of variation (CV) is for counts ranging from 114 to 3,592 cells per individual and is given as a mean for sample sizes >1.

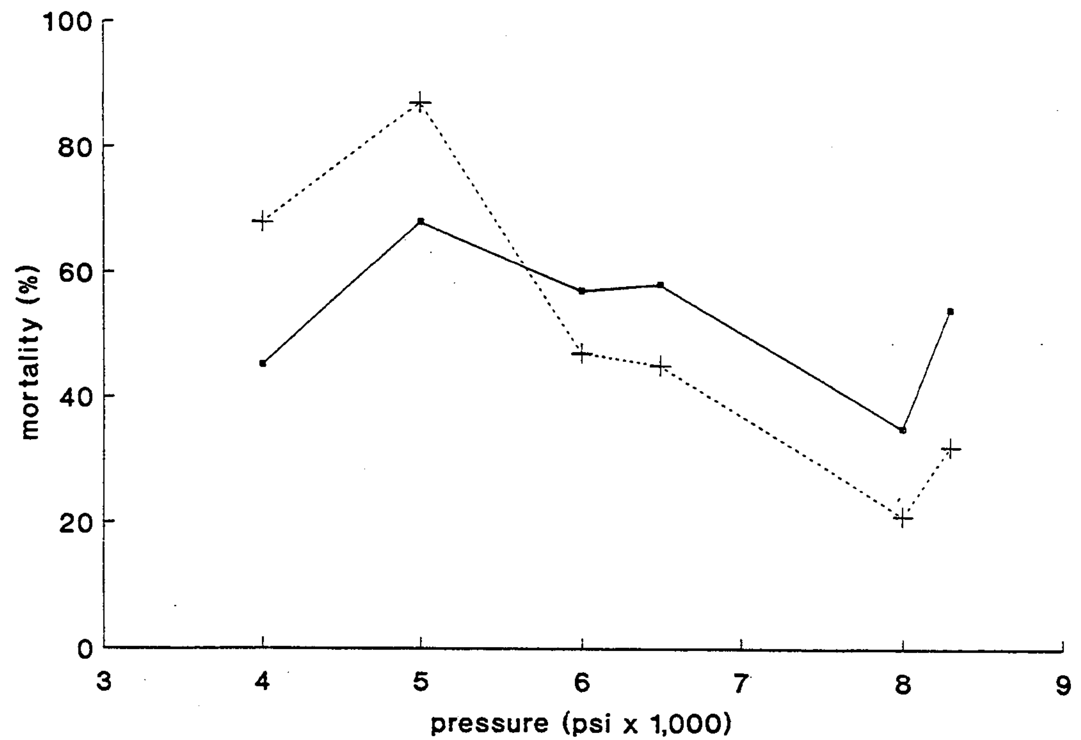
Treatment	Number examined	Ploidy		Mean pg DNA (CV)	
		2N	3N	2N	3N
Control	41	41	0	1.97(4.3)	-
4,000 p.s.i. for 3 min	5	4	1	1.92(3.0)	2.99(2.0)
5,000 p.s.i. for 3 min	3	0	3	-	2.99(2.3)
6,000 p.s.i. for 3 min	28	8	20	1.96(4.5)	2.99(3.5)
6,500 p.s.i. for 3 min	1	0	1	-	3.25(2.6)
8,000 p.s.i. for 1 min	19	0	19	-	3.15(3.3)
8,300 p.s.i. for 1 min	5	0	5	-	3.03(2.3)

The relatively low mortality in the 8,000 p.s.i. treatment (Figure 1) may have been fortuitous, but higher pressure, in general, was not positively correlated with mortality.

Because 100% triploidy induction was obtained, high pressures near 8,000 p.s.i. for a duration of 1 minute appear best for triploid production in largemouth bass. Pressures as low as 5,000 p.s.i. for 3 minutes also produced 100% triploids, but the sample size was very small. The next higher pressure treatment, 6,000 p.s.i. for 3 minutes, produced only 71% triploids. Although there surely are other combinations of pressure and duration that will produce a large percentage of triploids, a pressure of 8,000 p.s.i. will produce 100% triploids and does not increase mortality. Even higher pressures (i.e., 8,300 p.s.i.) also produce triploids but are not necessary.

These experiments have shown triploidy can be induced in largemouth bass but give no indication of ultimate physiological effects. Research is continuing on pertinent aspects such as relative growth rates and reproductive viability.

FIGURE 1. Percent pre-hatching mortality of largemouth bass eggs treated with hydrostatic pressure (treatment = solid line) and groups of untreated eggs (control = dashed line) associated with each treatment group. Sample sizes for treatment and control groups ranged from 100 to 500 individuals.



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