Induction of Polyploidy with Hydrostatic Pressure in Striped Bass, White Bass, and Their Hybrids

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Abstract: Eggs of striped bass and white bass were subjected to hydrostatic pressures of 5,000, 6,000, 7,000, and 8,000 PSI for a duration of 5 minutes (2.5 minutes for 8,000 PSI) at 29, 45, or 55 minutes after fertilization. The most effective pressures for producing tetraploidy in white bass were 5,000 and 6,000 PSI and for striped bass 7,000 and 8,000 PSI. Late in the spawning season, only 45- and 55-minute treatments were effective for producing tetraploidy. The relative hatch of eggs subjected to hydrostatic pressure for tetraploid induction was 55% of controls. Original and reciprocal striped bass × white bass hybrid eggs were subjected to hydrostatic pressures of 6,000, 7,000, and 8,000 PSI, applied 2 to 7 minutes after fertilization for durations of 2 to 5 minutes. Several treatments produced triploids; however, 8,000 PSI was the most effective pressure (50% triploid induction) when initiated 2 or 7 minutes after fertilization for a duration of 3 to 5 minutes. The relative hatch of eggs treated for triploid induction was 63% of controls.

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Production of polyploid fish has received considerable attention in recent years, and several techniques for inducing polyploidy have been reported (Purdom 1983). Hydrostatic pressure was used to induce triploidy in rainbow trout, *Salmo gairdneri* (Lou and Purdom 1984), and in Atlantic salmon, *Salmo salar* (Benfey and Sutterlin 1984). Functionally sterile triploid grass carp, *Ctenopharyngodon*

idella, have been produced by both hydrostatic pressure and thermal shock of fertilized eggs (Cassani and Caton 1986). Cassani and Caton (1986) found that hydrostatic pressure produced more consistent and effective results than thermal shock when attempting to induce polyploidy in grass carp. Rezk (1988) also concluded that hydrostatic pressure induced polyploidy in hybrid catfish more effectively than thermal shock.

Sterility of triploids (Wolters et al. 1982, Cassani and Caton 1986) may allow a species or hybrid to be stocked in locations where fertile species or hybrids could contaminate native gene pools or where a reproducing species is undesirable. Reciprocal striped bass, *Morone saxatilis,* × white bass, *M. chrysops,* hybrids are a desirable sport fish; however, their fertility (Bayless 1972, Avise and Van Den Avyle 1984) allows the potential for backcrossing and contaminating the parent species. Triploid fish may also grow faster since metabolic energy required for gonad and gamete production can be used for somatic growth. Wolters et al. (1982) found that growth rates and food conversion in triploid channel catfish, *Ictalurus punctatus,* were significantly higher than diploid controls in tanks.

If fertile tetraploids can be produced and crossed with diploids, the progeny should consist of sterile triploids. Inducement of triploidy by this natural manner is likely to be more efficient for producing triploids than shock treatments. Tetraploidy has been induced in rainbow trout embryos subjected to thermal, chemical, or hydrostatic pressure shock just prior to first cleavage (Thorgaard et al. 1981, Refstie 1983, Chourrout 1984).

Chourrout et al. (1986) have found that tetraploid rainbow trout males can be fertile and successfully fertilize diploid eggs. Fertility of those males varied, possibly due to larger sperm size of tetraploids and variation in size of micropyles of eggs from diploid females. Tetraploid males crossed with diploid females resulted in 96% triploid progeny (Chourrout et al. 1986). The remaining progeny were diploid or aneuploid.

Although hydrostatic pressure has been used to consistently produce polyploids in several species, the amount of pressure, timing of treatment, and treatment duration must be determined for each species. Our objective was to determine parameters of hydrostatic pressure treatments necessary to produce tetraploid striped bass and white bass and to produce triploid striped bass-white bass reciprocals. If triploid hybrid bass can be produced by hydrostatic pressure or by crossing tetraploids with diploids, and if triploids are sterile, triploid hybrid bass might be utilized in sport fisheries management or aquaculture without the danger of their backcrossing to their parents or producing F_2 hybrid bass.

Methods

Brood fish were collected by electrofishing and transported to holding facilities at the Jack D. Bayless Fisheries Research Hatchery, St. Stephen, South Carolina. Males were placed in a 4,477-liter rectangular tank and females were placed in 37,374-liter circular tanks maintained at 16.5° to 19.5°C. All female brood fish,

regardless of abdomen distension, were injected intramuscularly with chorionic gonadotropin at 275 to 300 IU/kg of body weight. Male white bass were also injected at the same dosage to increase milt volume. Ovulation was predicted by microscopic examination of an egg sample taken between 20 and 24 hours after injection. Ovulation was confirmed by observance of freely flowing eggs resulting from manual pressure on the abdomen.

Replicate batches of eggs obtained from individual striped bass or white bass females were fertilized with milt from the same species and subjected to pressure treatments of 5,000, 6,000, 7,000, and 8,000 pounds per square inch (PSI) at 29, 45, and 55 minutes after fertilization for 5 minutes (2.5 minutes for 8,000 PSI) for induction of tetraploidy. First cell division was approximately 60 minutes after fertilization at 16.5°C. The timing of these pressures corresponded to those that had been successful in other species of fish (Chourrout 1984, Myers 1985, Rezk 1988). Eggs from each female were divided, used in all treatments, and fertilized by the same males. Replicate batches of eggs from each species were also fertilized with milt from the opposite species and treated at 6,000, 7,000, and 8,000 PSI at 2 to 7 minutes after fertilization (Wolters et al. 1982, Cassani and Caton 1986) for 3 to 5 minutes for induction of triploidy. The same males were utilized in each replicate. Fertilization was accomplished with the dry method (Bayless 1972) to ensure administration of the pressure treatments at a known time after fertilization (addition of water). Untreated controls were produced for each replicate. Two to 4 replicate batches of eggs from different females were used per treatment.

The pressure chamber consisted of a stainless steel cylinder 4.4 cm in diameter and 30.5 cm long with an opening 28.0 cm in length and 2.1 cm in diameter. A 12.5-cm brass piston fitted with a rubber flange on the bottom formed an air-tight seal. The piston was drilled and fitted with a pressure relief valve. The cylinder with piston in place held approximately 120 ml.

Approximately 60 ml of fertilized eggs and 60 ml of water were poured into the chamber at a specified time after fertilization. The piston was inserted with the relief valve open and was forced into the cylinder by hand pressure until air was purged and relief valve closed. A hand-operated hydraulic press generated the thrust to produce treatment pressures and could apply approximately 2,000 PSI/sec at a standard rate of operation. Treatment pressures were calculated from pressure applied by the press as measured by a certified pressure gauge attached to the press. Duration of treatment excluded the 2.5 to 4 seconds needed to generate treatment pressure.

After treatment, eggs were incubated in a modified McDonald hatching jar (Bayless 1972). After 24 hours, approximately 150 eggs were randomly collected from controls and from each treatment for microscopic examination of embryonic development. Percentage of developing embryos was obtained and used to estimate percent hatch for the treatment and control groups.

Fry 1 to 5 days old and eggs 40 hours old were transported in sealed plastic bags containing water and oxygen (Bayless 1972) to Auburn University for determination of ploidy level. Ploidy of larvae or embryos was determined by either

karyotyping (Wolters et al. 1982), silver staining of nucleolar organizers (Phillips et al. 1986), or by modifying both of these techniques and simultaneously applying them to an individual embryo. When karyotyping was utilized, 10 or more methaphase spreads were examined per individual, and when silver staining of nucleolar organizers (NORs) was utilized, 100 cells were examined per individual. An average of 9 individuals were examined for triploid induction treatments and 7 for tetraploid induction treatments.

Percentage tetraploid induction of white bass and striped bass was compared with Chi-square. Correlations were computed for the relationships between treatment pressure and percentage tetraploid induction in white bass and striped bass.

Results and Discussion

Diploid number for *Morone* bass in this experiment was 48 which is in agreement with results of Rachlin et al. (1978) found for striped bass in the Hudson River. Triploids had 72 chromosomes and tetraploids had 96 chromosomes. All control diploids exhibited 1 or 2 NORs in each cell. The number of NORs in diploids of various species can vary from 2 to 6, but is usually 2 (Gold 1984). Triploids exhibited 1, 2, or 3 NORs in each cell. Tetraploids exhibited 1, 2, 3, or 4 NORs in each cell.

Silver nitrate does not stain NORs genetically inactive in the previous interphase, cells in late prophase I-metaphase II, or those in meiosis (Howell 1982, Schmid et al. 1982, Satya-Prakash and Pathak 1984). Phillips et al. (1986) found that by examining 10 or more cells per individual, ploidy level in rainbow trout could be accurately determined and that NORs were found at higher frequencies in fry than older fish. When we simultaneously karyotyped and silver-stained an individual, the number of chromosomes observed and number of NORs were in agreement.

Survival of control eggs from white bass was 46% and from striped bass 51%. There were 1,000 white bass eggs/ml and 300 striped bass eggs/ml, allowing treatment of 60,000 white bass or 18,000 striped bass eggs in the pressure chamber at one time. Hatch of eggs treated for tetraploid induction averaged 45% lower than controls and ranged from 12% to 90% lower than controls (Table 1). Hatch of eggs treated for triploid induction averaged 37% lower than controls (Table 2) and ranged from 0 to 80% lower than controls. Survival was sufficient that adequate numbers of *Morone* eggs would survive treatments of hydrostatic pressures for practical hatchery applications.

Both tetraploid white bass and striped bass were produced (Table 1). Eight of 10 treatments for white bass and 7 of 11 treatments for striped bass resulted in some tetraploid production (Table 1). Treatments at 5,000 and 6,000 PSI were the most successful for white bass, and treatments at 7,000 and 8,000 PSI were the most successful for striped bass (Tables 1, 3). The correlation between treatment pressure and percent tetraploidy was -0.98 and 0.72 for white bass and striped bass, re-

Table 1. Treatment parameters, percent tetraploidy, and hatch of striped bass and white bass embryos treated with hydrostatic pressure at a water temperature of 16.5° C. All treatment durations were 5 minutes except 2.5 minutes was used for 8,000 PSI treatments.

Species	Pressure (PSI)	Initiation of shock (min post-fertilization)	*N*	% tetraploidy	Hatch % relative to control
White bass	5,000	29	2	100	53
		45	3	100	39
		55	6	83	47
	6,000	29	10	30	71
		45	3	67	10
		55	8	50	76
	7,000	29	2	50	76
		45	3	0	10
		55	8	25	37
	8,000	55	6	0	60
Striped bass	5,000	29	8	0	88
		45	4	0	88
		55	4	25	79
	6,000	29	15	0	58
		45	4	25	49
		55	5	0	32
	7,000	29	20	55	85
		45	6	50	65
		55	12	25	60
	8,000	45	4	25	38
		55	3	33	40

Table 2. Treatment parameters, percent triploidy, and hatch of reciprocal striped-white bass hybrid embryos treated with hydrostatic pressure at a water temperature of 16.5° C.

Hybrid	Pressure (PSI)	Initiation of shock (min post-fertilization)	Treatment duration (min)	*N*	% triploidy	Hatch % relative to control
White x striped (reciprocal)	6,000	4	2	10	20	70
	7,000	4	2	5	0	100
Striped x white (original)	7,000	2	5	8	0	58
		4	3	10	20	20
		6	5	11	9	84
	8,000	2	4	15	53	63
		2	5	10	30	100
		6	5	8	0	77
		7	3	11	46	54
		7	4	1	100	29

Table 3. Polyploid induction rates in *Morone* bass for treatments pooled by pressure, initiation of pressure or by genotype.

	% Tetraploidy							
	Pressure (PSI)				Initiation of pressure (minutes after fertilization)			
Genotype	5,000	6,000	7,000	8,000	29	45	55	Total
White bass	91	43	23	0	43	56	39	43
Striped bass	6	4	45	29	25	28	21	25

	% Triploidy						
	Pressure (PSI)			Initiation of pressure (minutes after fertilization)			
	6,000	7,000	8,000	2	4	6	7
Hybrids	20	9	49	33	16	5	50

spectively. There was no trend for success as related to timing of initiation of pressure, 29, 45, and 55 minutes, which should have corresponded to karyokinesis through cytokinesis. Percentage tetraploid induction was not different ($P > 0.05$) between the 2 species (Table 3).

Triploid *Morone* hybrids were observed at all tested pressures (Table 2). The highest pressure, 8,000 PSI, was more successful, 49% triploid induction, than 6,000 and 7,000 PSI, 20% and 9% triploid induction, respectively (Tables 2, 3). Initiation of pressures at 2 or 7 minutes after fertilization produced the largest percentages of triploids (Tables 2, 3). Treatments at 8,000 PSI 2 or 7 minutes after fertilization resulted in approximately 50% triploid induction.

Production of tetraploid white and striped bass was effective. Viability may be low in tetraploids (Myers 1985, Rezk 1988) and needs to be evaluated in juvenile and adult tetraploid *Morone*. Tetraploid rainbow trout are fertile (Chourrout et al. 1986) and if tetraploid *Morone* are fertile, the mating of tetraploids with diploids can be evaluated for efficiency of natural triploid production.

Rates of triploid induction by forcing retention of the second polar body were low in this experiment compared to near 100% triploid induction in other species (Wolters et al. 1982, Cassani and Caton 1986). If triploid induction is not 100%, screening of all individuals would be required to prevent introduction of a fertile diploid hybrid into the natural environment. Rate of triploid induction was increasing with increasing pressure, and higher levels of pressure should be evaluated for induction of triploidy in hybrid *Morone*. Production of triploids in this experiment will allow the evaluation of viability, growth, hook-and-line vulnerability, and fertility in triploid *Morone* hybrids.

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