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Induced Gynogenesis in Black Crappie

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Abstract.—This study reports the results of initial experiments on induced diploid gynogenesis in black crappie *Pomoxis nigromaculatus*. White bass *Morone chrysops* were an effective sperm donor for gynogenetic experiments with black crappies. White bass spermatozoa fertilized black crappie eggs, but hybrid larvae were not viable and died after hatching. In a series of experiments, we determined the ultraviolet (UV) light dose required to inactivate the sperm genome and the heat shock parameters needed to restore diploidy. Black crappie eggs were inseminated with white bass sperm irradiated with UV dosages ranging from 25 to 2,500 J/m^2. A typical "Hertwig effect" in the yield of hatched larvae was observed with dosages greater than 100 J/m^2. The genetic inactivation of paternal chromosomes was confirmed by flow cytometry measurement of DNA content in larval cells. Larvae resulting from sperm irradiated at a dose of 1,000 J/m^2 were haploid. Time durations of mitotic interval (τ_0) at different temperatures for black crappies have been determined to standardize data on heat shock timing in experiments with different preshock temperatures. The resultant regression equation ($\tau_0 = 70.96 - 2.32T$, where T is water temperature) was used for calculation of the value of τ_0 for any water temperature observed in the experiments. Application of heat shock was effective for suppression of the second meiotic division in eggs and resulted in morphologically normal diploid gynogenetic larvae. The effectiveness of shock was dependent on its parameters. The best yield of diploid gynogens (8.3% from the initial number of eggs) was observed after application of 37°C shock and 1.5-min duration at 0.05 τ_0 after insemination. Future experiments will be directed to increase the effectiveness of heat shock by further optimization of its parameters.

The black crappie *Pomoxis nigromaculatus* and white crappie *P. annularis* are popular game fishes in the United States and have potential as aquaculture species. One of the main obstacles to successful management of their populations in ponds and small impoundments and to the commercial rearing of these fishes involves their high rate of reproduction, which leads to overcrowding and subsequent stunting of individuals (USDA 1983; Martin 1988; Hooe 1991). Several approaches to solve this problem have been investigated. Methods to control excessive recruitment by stocking predators (largemouth bass *Micropterus salmoides*; Gabelhouse 1984) or by chemical and mechanical removal (Rutledge and Barron 1972; Hanson et al. 1983) have been tested. It has been suggested that the rate of reproduction could be decreased by the rearing of interspecies hybrids (Hooe et al. 1994) or triploid fish (Baldwin et al. 1990; Parsons 1993; Parsons and Meals 1997). Another possible solution is the production of monosex crappie progenies by hormonal sex reversal.

Sex reversal may be used in two different ways for creating monosex populations (Donaldson 1996). The direct method for producing monosex populations involves the hormonal treatment of all reared fish during period of sex differentiation. Recently Al-ablani and Phelps (1997) described the first successful attempt to masculinize black crappies by androgen treatment. An indirect (genetic) method involves crossing normal and sex-inverted fish of the same genotypic sex. In the case of female homogamety (genotype of females, XX; genotype of males, XY), which have been revealed in many aquaculture species, genetic sex regulation involves crossing normal XX females with sex-inverted XX males.

The first step for development of genetic sex regulation frequently includes developing techniques for producing gynogenetic progenies. The sex ratio in gynogenetic progenies provides information on the chromosomal sex determination

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mechanism in a given species; in case of female homogamety, gynogenetic progenies consist of only females. Also, it is convenient to use all-female gynogenetic progenies (instead of normal bisexual progenies) for sex inversion experiments because the appearance of any males in androgen-treated groups will indicate successful sex inversion. Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several aquaculture species: common carp *Cyprinus carpio* (Gomelsky 1986; Gomelsky et al. 1994), grass carp *Ctenopharyngodon idella* (Shelton 1986), silver carp *Hypophthalmichthys molitrix* (Mirza and Shelton 1988), tench *Tinca tinca* (Linhart et al. 1995), and others.

In the present paper, we report the results of our initial experiments on induced gynogenesis in black crappies. We describe also our methodological approach for step-by-step development of a gynogenetic technique for a new species. Heterologous sperm, instead of sperm from the same species, is most useful in gynogenetic experiments because nonviability of diploid hybrids indicates that survivors are of gynogenetic origin (Chourrout 1987). Consequently, our first objective was to identify species compatible as a sperm donor to induce gynogenetic development in black crappie eggs. Further experiments have investigated the optimal dose of ultraviolet (UV) irradiation of male chromosomes and effective parameters of heat shock for suppression of second meiotic division in eggs. We also determined the values of the temperature-dependent index of mitotic interval (τ_0; Dettlaff and Dettlaff 1961; Dettlaff 1986) for black crappie in a special experiment of this study. This index permits comparing the data on shock timing obtained in experiments with different preshock water temperatures (Gomelsky et al. 1989; Cherfas et al. 1990; Shelton and Rothbard 1993; Lin and Dabrowski 1998; Mims and Shelton 1998).

Methods

The experiments were conducted at the Aquaculture Research Center (ARC) of Kentucky State University in 1997 and 1998. Black crappie broodstock were collected from Minor Clark Coldwater Fish Hatchery, and Taylorsville Lake, Kentucky, and William H. Donham State Fish Hatchery, Arkansas. Fish were transported to ARC and held in a 200-m^2 earthen pond until spawning season or used immediately for spawning induction.

Sexually mature black crappies were sorted by sex, paired (one male and one female), and placed into separate 115-L aquaria. Male black crappies were given a single intramuscular injection of luteinizing hormone releasing hormone analog ($LHRH_a$; 50 μg/kg of body weight) or human chorionic gonadotropin (hCG, 1,000 IU/kg). Similar injections were given to males of white bass *Morone chrysops*, walleye *Stizostedion vitreum*, common carp *Cyprinus carpio* and blue sucker *Cycleptus elongatus*. Females were given a total dose of $LHRH_a$ (100 μ/kg) or hCG (1,000 IU/kg) in two injections (0.1 and 0.9, respectively, of the total dose) separated by a 12 h interval. We closely observed fish in each aquarium, and when spawning behavior was first observed, the female was removed, and the eggs were stripped immediately. Black crappie males did not release sperm by stripping. Therefore males were sacrificed, and their testes were dissected and washed with a 0.85% saline solution. The sperm suspension was used for egg insemination. Sperm from white bass, walleye, common carp, and blue sucker males was collected by stripping.

Identification of suitable sperm donor (experiment 1).—Black crappie eggs (taken from one female) were inseminated with the sperm of black crappies (control), white bass, walleyes, common carp, and blue suckers. The inseminated eggs were spread evenly on glass petri dishes (about 1,000–1,500 eggs/dish), which were then placed individually in 0.5-L bowls for incubation. The total number of eggs, fertilization rate, total number of hatched larvae, and the number of morphologically normal larvae were recorded for each cross. Fertilization rate was determined microscopically during initial cleavage stage (4–32 blastomeres).

Determination of optimal dose of ultraviolet irradiation (experiment 2).—Eggs taken from one black crappie female were inseminated with white bass sperm (which appeared to be the best sperm donor according to results of experiment 1) irradiated at 0 (control), 25, 50, 100, 250, 500, 1,000, 2,000, and 2,500 J/m^2. Before irradiation, sperm was diluted (1:10) in a 0.85% NaCl solution to improve UV penetration. This salt concentration was used earlier (Gomelsky et al. 1998) for dilution of sperm of striped bass *Morone saxatilis* in experiments on induced gynogenesis in white bass. A FisherBiotech UV microprocessor-controlled Crosslinker (FB-UVXL-1000; Fisher Scientific) was used for sperm irradiation. This model has five 8-W, 254-nm UV bulbs and is equipped with a system for direct metering of total energy per unit area. The 6-cm glass petri dish containing 2 mL of dilute sperm was placed inside the Cross-

linker, and the target dosage was delivered automatically. The uniform irradiation of spermatozoa was achieved by placing the Crosslinker on a shaker table to keep the sperm in motion during treatment. Total number of eggs, the fertilization rate, and total number of hatched larvae were recorded for each dose.

Determination of τ_0 for black crappie (experiment 3).—The τ_0 was determined according to the technique described earlier (Shelton and Rothbard 1993; Shelton et al. 1997). In brief, the time when embryos reached the stages of two, four, and eight blastomeres were recorded at four different temperatures (18, 20, 22, and 24°C) in three replicate crosses of one female and one male. Inseminated eggs were incubated in petri dishes placed in tanks equipped with thermostats. The values of τ_0 were calculated from the average time between two consecutive cleavages (two to four and four to eight cells). The embryos were observed under a microscope; the embryological stage was fixed based on about the first 10% of the eggs that cleaved. The relationships between mean mitotic interval and water temperature were examined by general linear model (GLM) procedures (SAS Institute 1985).

Production diploid gynogens by heat shock (experiments 4–6).—Black crappie eggs were inseminated with UV-irradiated white bass sperm (dosage 1,000 J/m^2, which appeared to be optimal, according to results of experiment 2) and were subjected to heat shocks of different regimes. In each experiment, the eggs from one female were used. Three different shock temperatures (35, 37, and 39°C) were tested in experiment 4, with a shock of 2-min duration applied 2.5 min after insemination. To evaluate the viability of triploid hybrids, the heat shock of 37°C was applied also to a portion of eggs inseminated with nonirradiated sperm. Experiments 5 and 6 were designed to evaluate different timing of heat shock applications. In experiment 5, a 2-min shock of 37°C was applied at 2, 3, 4, or 5 min after insemination. In experiment 6, a 1.5-min shock was applied at 1, 3, 5, and 9 min after insemination. Timing of heat shock application was expressed both in minutes and in τ_0, which was calculated for each preshock temperature according to data obtained in experiment 3. Total number of eggs, the number of hatched larvae, and the number of morphologically normal larvae were recorded. To estimate heat shock damage, "hatchability" was determined as the ratio of number of hatched larvae to the number of treated eggs in each portion. The yields of morphologically normal (diploid) larvae from both the total number of hatchlings and the number of treated eggs served as main indices of shock effectiveness. Control haploid gynogenetic progenies were obtained in each experiment by insemination of eggs with irradiated sperm but without shock application. Control diploid hybrid progenies by insemination of eggs with nonirradiated white bass sperm and without heat shocks were also produced. Differences in the percentages of morphologically normal larvae among gynogenetic progenies were tested for significance by *G*-tests (Sokal and Rohlf 1981).

Ploidy determination.—Flow cytometry measurement of cellular DNA content was used to verify the ploidy of progenies. Twenty morphologically normal gynogenetic larvae from shock-treated groups, as well as 20 hatched abnormal larvae from both the control haploid gynogenetic progeny group (irradiated sperm but without shock) and control diploid hybrid progeny group (no shock and no sperm irradiation) of experiment 6 were analyzed. Flow cytometry technique was similar to that described by Lin and Dabrowski (1996), with some modifications. Briefly, the yolk sac was dissected from newly hatched fry, and the embryos were fixed in 5% DMSO and kept frozen until analysis. Embryos were transferred into 12 × 75-mm sterile plastic tubes with snap cap (Fisher Scientific, Pittsburgh, Pennsylvania) containing 800 μL propidium iodide stain and 10 μL of blood from rainbow trout *Oncorhynchus mykiss* (internal standard). The solution was incubated overnight at 4°C, gently syringed, and filtered using 60-μm Nytex filters. Flow cytometry analysis was performed on a Coulter EPICS Elite flow cytometer (Coulter Corporation, Miami, Florida) equipped with a 488-nm, 15-mW air-cooled Argon laser. A minimum of 10,000 gated cells were collected at the rate of 500 events/s. Propidium iodide signal was measured with a 610-nm-long pass transmission filter and represented in linear mode. Single parameter statistics on sample and internal standard peak positions were generated by means of a Standard Elite Workstation Software. Relative DNA was expressed as the ratio of the DNA content of embryo cells to that found in rainbow trout red blood cells (internal standard).

Results

Fertilization rate and survival of hybrid embryos obtained after insemination of black crappie eggs with the sperm of four different species (experiment 1) are given in Table 1. The female black

TABLE 1.—Fertilization and hatching rates of black crappie eggs after insemination with sperm of difference species.

Cross[a]	Number of eggs	Number of fertilized eggs	Fertilization rate (%)	Hatchlings		Normal larvae
				Number	Percent[b]	
♀BC × ♂WB	1,635	966	59.1	900	93.2	0
♀BC × ♂WE	1,641	927	56.5	165	17.8	0
♀BC × ♂CC	1,510	9	0.6	0	0	
♀BC × ♂BS	~1,500	0	0			
♀BC × ♂BC[c]	1,088	924	84.9	910	98.5	828

[a] BC = black crappie, WB = white bass, WE = walleye, CC = common carp, BS = blue sucker.
[b] From the number of fertilized eggs.
[c] Control.

crappie × male black crappie cross (control) resulted in high fertilization rate (84.9%) and hatchability (98.5%), and most of the hatched larvae (91%) were morphologically normal. Blue sucker sperm failed to fertilize the black crappie eggs; whereas, some eggs inseminated with common carp sperm initiated embryonic development. Rates of fertilization for eggs inseminated with white bass or walleye sperm were similar at 59.1% and 56.5%, respectively. However, black crappie × white bass hybrids showed 93% hatchability, and all larvae had various morphological abnormalities. Shortening of the bodies and deformed heads were the most frequent abnormalities. All black crappie × white bass larvae died within several days after hatching. Black crappie eggs fertilized with walleye sperm usually died during embryonic development, and larvae that hatched (18%) had abnormalities and soon died. According to the results of experiment 1, it was concluded that white bass would be the best sperm donor for gynogenetic experiments with black crappies.

The results of incubation of black crappie eggs inseminated with white bass sperm irradiated at different UV doses (experiment 2) are presented in Table 2 and Figure 1. Fertilization rate decreased with increase in dosage. The percentage of hatched eggs decreased for doses from 0 to 100 J/m², presumably because of damaged male chromosomes, but with further increases in dose, hatching rate of fertilized eggs increased. The majority of embryos obtained at doses of 500–2,500 J/m² developed a typical haploid syndrome which was characterized mainly by the shortening of the body and tail curvature. At doses greater than 500 J/m², the percentage of hatched larvae stabilized at 81–85% of the number of fertilized eggs. The dose of 1,000 J/m² was used in further experiments because, based on the result of experiment 2, it provided genetic inactivation of sperm and maintained better fertilization rate in comparison with higher doses.

TABLE 2.—Fertilization and hatching rates after insemination of black crappie eggs with white bass sperm exposed to different dosages of ultraviolet (UV) radiation.

UV radiation (J/m²)	Number of eggs	Number of fertilized eggs	Fertilization rate (%)	Hatchlings		Normal larvae
				Number	Percent[a]	
0	660	380	57.6	316	83.2	0
25	288	157	54.5	114	72.6	0
50	552	242	43.8	159	65.7	0
100	603	250	41.5	92	36.8	0
250	707	175	24.8	102	58.3	0
500	450	100	22.2	78	78.0	1
1,000	718	195	27.2	166	85.1	0
1,500	534	102	19.1	88	86.3	0
2,000	408	21	5.1	17	81.0	0
2,500	444	35	7.9	25	83.3	0

[a] From the number of fertilized eggs.

The mitotic interval (τ_0) decreased with increasing temperature (experiment 3; Figure 2). Mean (calculated) mitotic intervals and standard deviations at different temperatures were 29.7 ± 0.8 min at 18°C, 24.5 ± 1.0 min at 20°C, 18.8 ± 1.4 min at 22°C, and 16.0 ± 0.7 min at 24°C. The obtained data were best described by a linear relationship between τ_0 and water temperature (Figure 2). The resultant regression equation ($\tau_0 = 70.96 - 2.32T$; T = water temperature) was used to calculate τ_0-values for preshock temperatures observed in experiments 4–6.

The results of experiment 4 (Table 3) showed that application of heat shock reduced embryo survival. With the increase of shock temperature there was a decrease in the yield of hatchlings from 14% at shock temperature of 35°C to 0.4% at a shock temperature of 39°C, compared with 37.5% in control haploid gynogenetic progeny obtained without shock. Heat shock was effective for suppression of the second meiotic division in eggs and resulted

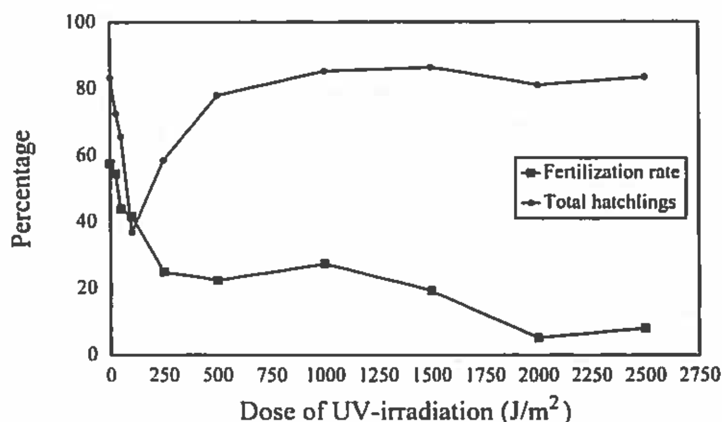

FIGURE 1.—Effect of sperm irradiation with ultraviolet (UV) light at various dosages on fertilization rate and yield of hatched larvae.

in the appearance of morphologically normal diploid gynogenetic larvae. Such larvae were viable and swam up after several days, in contrast to abnormal larvae (having haploid syndrome) which soon died. The most effective shock temperature was 37°C; 55.2% of the hatched larvae (3.4% from the number of eggs) were normal. A temperature of 35°C was not sufficiently high enough to suppress second meiotic division and resulted in appearance of only two normal larvae from 58 hatchlings (3.4%), while a temperature of 39°C was mostly lethal. No normal larvae developed in control gynogenetic and hybrid progenies obtained without shock application, as well as in hybrid progeny subjected to heat shock of 37°C.

In experiment 5 (Table 4) the highest yield of normal larvae both from number of hatchlings (50%) and from number of eggs (3.3%) was observed after shock application at 2 min after insemination. The shock application 5 min after insemination decreased the percent yield of normal larvae among hatchings to 19%, although the yields of normal larvae from the number of eggs after shock application at 2 and 5 min (3.3% and 2.5%, respectively) did not differ significantly.

The more definitive data on the dependence of shock effectiveness from its timing was obtained in experiment 6 (Table 4). Heat shock applied at 1 and 3 min after insemination resulted in similar yields of normal larvae from number of hatchlings

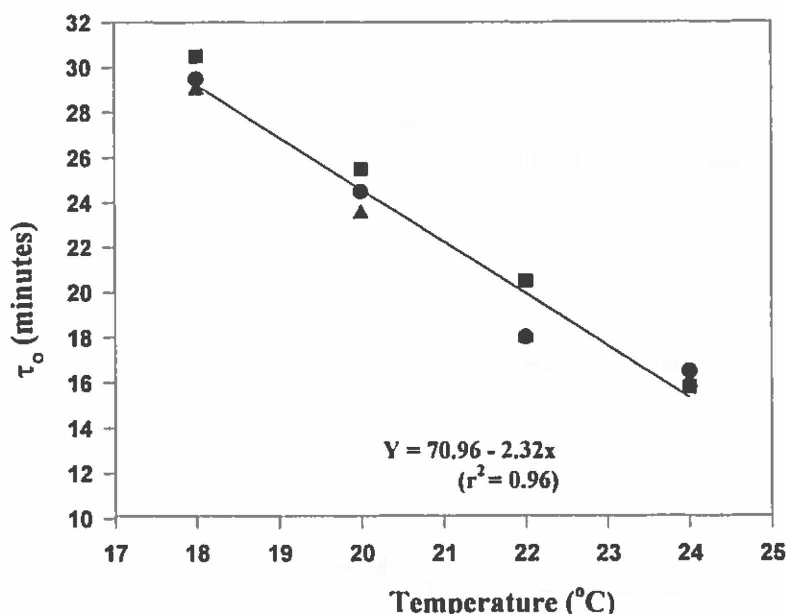

FIGURE 2.—Duration of mitotic interval (τ_0) in black crappies as a function of temperature. Each kind of designation (circle, triangle, or square) represents a cross of one female and one male.

TABLE 3.—Hatching rate and yield of normal larvae after heat-shock treatments applied to black crappie eggs inseminated with white bass sperm.

Type of progeny	Heat shock application[a] Min	Heat shock application[a] τ_0	Shock temperature (°C)	Number of eggs	Hatchlings Number	Hatchlings Percent[b]	Number of normal larvae	Yield of normal larvae (%)[c] From number of hatchlings	Yield of normal larvae (%)[c] From number of eggs
Gynogens[d]	2.5	0.09	35.3	413	58	14.0	2	3.4 y	0.5 y
			37.0	464	29	6.3	16	55.2 z	3.4 z
			39.2	522	2	0.4	1	50.0 zy	0.2 y
	None			635	238	37.5	0		
Hybrids[e]	2.5	0.09	37.0	398	38	9.5	0		
	None			492	249	50.6	0		

[a] Time after insemination; pretreatment water temperature was 18.4°C, τ_0 = 28.3 min.
[b] From the number of eggs in portion.
[c] Values within a column without a letter in common are significantly different ($P < 0.01$).
[d] Obtained with irradiated sperm.
[e] Obtained with nonirradiated sperm.

(37.4 and 35.3%, respectively). However, due to better survival observed at 1 min after insemination (22.1% of hatchlings) the yield of normal larvae from the number of eggs was significantly ($P < 0.01$) higher (8.3%). Application of heat shock at 5 min after insemination yielded only two normal larvae (7.7% of the hatchlings and 0.7% of the eggs). Heat shock applied at 9 min was completely ineffective for suppression of second meiotic division in eggs and did not result in appearance of normal larvae despite relatively high embryo survival (18.5% of hatchlings).

Flow cytometry revealed that all morphologically normal gynogenetic larvae ($N = 20$; experiment 6), as well as all normal hybrid black crappie × white bass larvae ($N = 20$), were diploid and had the amount of DNA equal to about 0.4 of that in the internal control (Figure 3A). Eleven of 19 larvae (one larva showed no DNA peak) from the control gynogenetic progeny, obtained by eggs inseminated with irradiated sperm (1,000 J/m^2) but without temperature shock, were haploid (about 0.2 of the DNA content in the internal control; Figure 3B). The other eight (42%) larvae were haploids with small amounts (from 6.6% to 15.1%, mean 11.4 ± 0.92%) of diploid cells (Figure 3C).

TABLE 4.—Hatching rate and yield of normal larvae after heat shock treatments applied to black crappie eggs inseminated with irradiated white bass sperm.

Type of progeny	Heat shock application[a] Min	Heat shock application[a] τ_0	Number of eggs	Hatchlings n	Hatchlings (%)	Number of normal larvae	Yield of normal larvae (%)[b] From no. of hatchlings	Yield of normal larvae (%)[b] From no. of eggs
Experiment 5 (shock temperature = 37° C, duration = 2 min)								
Gynogens[c]	2	0.08	300	20	6.7	10	50.0 z	3.3 z
	3	0.12	586	26	4.4	7	26.9 zy	1.2 y
	4	0.16	588	47	8.0	15	31.9 zy	2.6 zy
	5	0.20	397	52	13.1	10	19.2 y	2.5 zy
	None		647	211	32.6	0		
Hybrids[d]	None		498	254	51.0	0		
Experiment 6 (shock temperature = 37° C, duration = 1.5 min)								
Gynogens[c]	1	0.05	411	91	22.1	34	37.4 z	8.3 z
	3	0.16	241	17	7.1	6	35.3 zy	2.5 y
	5	0.27	275	26	9.5	2	7.7 y	0.7 y
	9	0.48	292	54	18.5	0		
	None		260	87	33.5	0		
Hybrids[d]	None		226	107	47.3	0		

[a] Pretreatment water temperature was 19.7° C (τ_0 = 25.3 min) in experiment 5 and 22.5° C (τ_0 = 18.8 min) in experiment 6.
[b] For each experiment, values within a column without a letter in common are significantly different ($P < 0.05$ for experiment 5, and $P < 0.01$ for experiment 6).
[c] Obtained with irradiated sperm.
[d] Obtained with nonirradiated sperm.

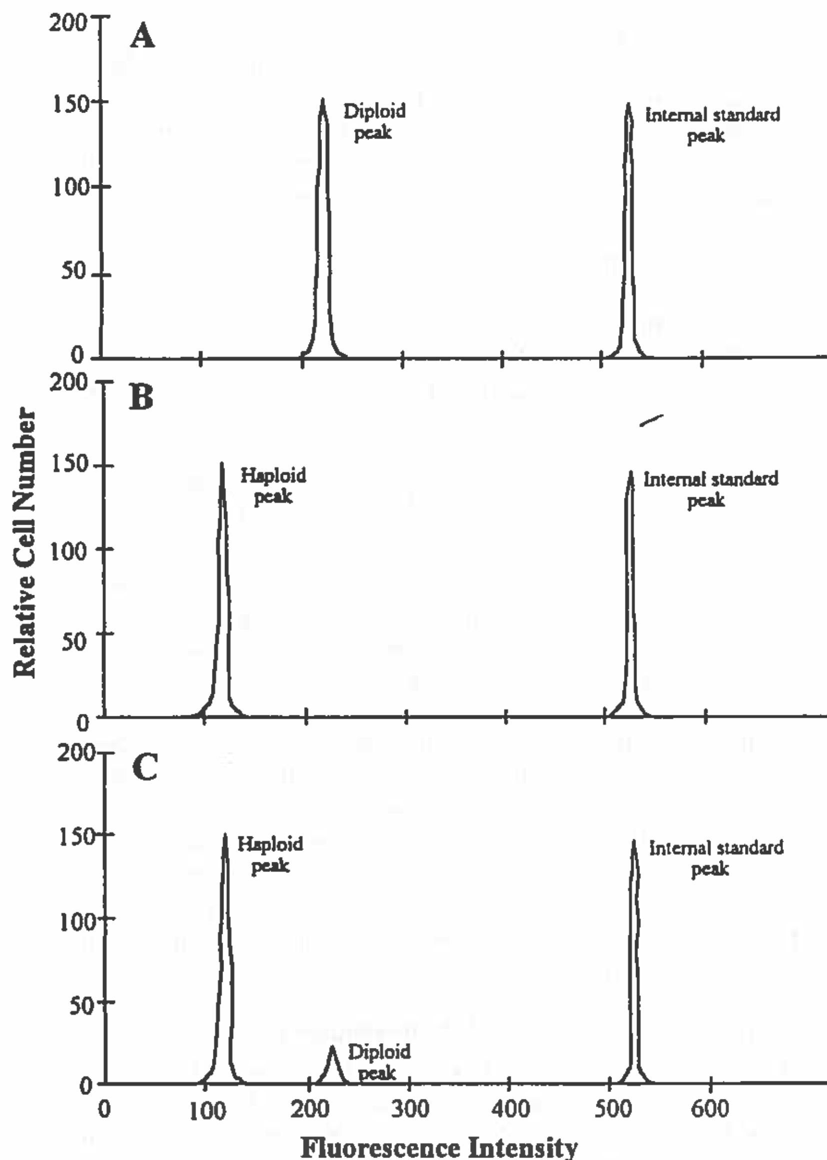

FIGURE 3.—Representative fluorescent distribution of (A) diploids (gynogenetic black crappies and black crappie × white bass hybrids), (B) haploids, and (C) haploids with some amount of diploid cells. Rainbow trout blood was used as an internal standard. The ratio between the haploid and diploid peaks in black crappie to that of rainbow trout was about 0.2 and 0.4, respectively.

Discussion

The results of experiment 1 demonstrated that white bass sperm might be suitable for induction of gynogenesis in black crappie: spermatozoa of white bass fertilized the crappie eggs, but the hybrid larvae were totally inviable and died soon after hatching. As was noted by Chourrout (1987), a heterologous sperm donor must provide inviability of not only diploid but also triploid hybrids. It was shown (experiment 4) that the shock, which successfully induced a high yield of normal gynogenetic larvae, did not result in occurrence of viable larvae in hybrid progeny that was obtained without sperm irradiation. This indicates that triploid hybrids of black crappie × white bass are also inviable and that survivors of progenies obtained with sperm irradiation and shock application are of gynogenetic origin.

The main indications of genetic inactivation of irradiated sperm were the "Hertwig effect" (i.e., paradoxical increase of embryo viability with increase of dosage; Thorgaard 1983; Chourrout 1987) and haploidy of hatched embryos obtained with sperm irradiated at high dosage. The Hertwig

effect was manifested as the increase in the relative number of hatched larvae at doses higher than 100 J/m^2 (experiment 2) and was (obviously) caused by gradual inactivation of male chromosomes. Stabilization of the percentage of hatched larvae at 81–85% after the 1,000 J/m^2 dose indicated inactivation of male chromosomes in all embryos. Because the heterologous sperm of white bass was used for insemination of black crappie eggs, the larvae obtained at all doses of irradiation were morphologically abnormal: at low doses, diploid hybrid larvae and at high doses, haploid black crappies were produced. However, because most (>80%) of black crappie × white bass hybrid larvae from control variant (i.e., without irradiation), as well as haploid larvae of black crappie, were capable of hatching, the Hertwig effect was clearly demonstrated. Flow cytometry measurement of DNA content verified the haploidy of larvae obtained after eggs inseminated with sperm irradiated at a dose of 1,000 J/m^2 and confirmed the genetic inactivation of male chromosomes. The appearance of one normal crappie gynogenetic larva obtained with sperm irradiation at a dosage of 500 J/m^2 may have resulted from spontaneous suppression of the second meiotic division.

The flow cytometry analysis revealed that about 40% of the analyzed haploid larvae had some amount of diploid cells. Earlier, Lin and Dabrowski (1996) described similar mosaicism with haploid embryos of muskellunge which had about 5% cells with doubled amount of DNA. To explain this phenomenon, the authors (Lin and Dabrowski 1996) suggested that haploids have an abnormal mitotic cycle with a delay at the interphase G2 stage (after DNA replication) and that the increased number of cells remaining at this stage was detected. It should be noted that both in Lin and Dabrowski (1996) and in the present study, diploids did not demonstrate any sign of an analogous G2 peak. The presence of some amount of diploid cells in haploid embryos may also be explained by possible somatic polyploidization, which reflects the instability of developmental processes in haploids, and results from sporadic suppression of mitotic cell divisions.

Results of experiments 4–6 showed that heat shock could suppress second meiotic division and yield viable diploid gynogens. Flow cytometry measurement of DNA content confirmed the diploidy of morphologically normal larvae in gynogenetic progenies. The effectiveness of shock was dependent on its parameters. Experiment 4 indicated that the optimal heat shock temperature was 37°C. The use of the values of mitotic index τ_0 determined in experiment 3 permitted comparison of the data on shock timing obtained in experiments 5 and 6 with different preshock water temperatures. Results of experiments 5 and 6 showed that heat shock applied at interval 0.05–0.16 τ_0 resulted in 27–50% of normal larvae from the total number of hatching. The application of heat shock within this interval essentially decreased the embryo survival, and the yield of normal larvae from the number of eggs was mainly determined by embryo hatchability. The best yield of normal gynogenetic larvae from number of eggs (8.3%) was observed in experiment 6 when 37°C heat shock of 1.5-min duration applied at 0.05 τ_0 after insemination provided relatively high (22.1%) postshock hatchability.

The results of present study indicate the possibility of producing diploid gynogenetic progenies in black crappie by using UV irradiation of sperm and application of heat shock at the time of the second meiotic division. Future experiments will be directed to the increase of yield of gynogenetic diploids by further optimization of heat shock parameters. Also it is planned to test the effectiveness of cold shock for suppression of second meiotic divisions in eggs. Earlier Baldwin et al. (1990) showed that cold shock was more effective than heat shock for inducing triploidy in white crappies.

Acknowledgments

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