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Induction of Polyploidy in Ictalurid Catfish and Comparative Performance of Diploid and Triploid Channel Catfish and Channel X Blue Catfish Hybrids.

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INDUCTION OF POLYPLOIDY IN ICTALURID CATFISH AND COMPARATIVE PERFORMANCE OF DIPLOID AND TRIPLOID CHANNEL CATFISH AND CHANNEL X BLUE CATFISH HYBRIDS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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Doctor of Philosophy

in

The School of Forestry, Wildlife and Fisheries

by

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ABSTRACT

Potential for genetic improvement of ictalurid catfish through induction of polyploidy has recently been the subject of several studies. This study provided indications of the feasibility of inducing polyploidy in ictalurids (channel catfish, Ictalurus punctatus, and hybrids with blue catfish, I. furcatus) by temperature or hydrostatic pressure shocks as well as the potential for improvement of growth and processing traits in triploid channel catfish x blue catfish hybrids.

One-cell embryos of ictalurid catfish were subjected to either temperature or hydrostatic pressure shocks for induction of triploidy or tetraploidy. Hydrostatic pressure was more consistent than cold-shocks for inducting triploidy with high survival of treated embryos. Pressures of 1300-1500 kg/cm^2 for 5 min at 5 min after fertilization induced 100% triploidy with 50% hatching percentage.

Hatch rates of embryos treated for induction of tetraploidy with hydrostatic pressure shocks were lower than those treated for induction of tetraploidy with heat shocks. Heat shock parameters previously reported as being effective for tetraploid induction in channel catfish did not induce tetraploidy in the present study.

The most effective heat shock and pressure shock treatments gave similar results, inducing approximately 30-70% tetraploidy. Treatments applied near first cell divisions (80 to 83 min post-fertilization) were more effective for producing tetraploidy than those applied earlier (43 to 75 min post-fertilization) in the cell cycle. Percent tetraploidy was higher when the fish were examined 2 days after fertilization than when examined at 3 months of age suggesting that induced tetraploid catfish are subviable. Diploid-tetraploid mosaics with compressed bodies and skeletal deformities were also produced.

It was hypothesized that triploid hybrids might have the advantages of heterosis as well as sterility caused by triploidy. No significant differences (α= .05) were detected in growth, feed efficiency, gonadosomatic index or dressout characteristics between diploid and triploid hybrids raised in 1.3 m diameter tanks, with the exception of the significantly higher (P = 0.0321) condition factor (K) of triploid hybrids. All diploid hybrids had normal gonadal development and histology. However, triploid hybrids had abnormal gonadal development with a single ovary present in 36% of triploid females. Both testes were present in all males. However, triploid hybrid males had abnormal histology with no sperm present in seminiferous tubules. Sex ratios did not differ significantly from 1:1 in either treatment. Survival for both treatment groups was 100% from age 14 months to 18 months.

No significant difference was found between diploid and triploid pond-grown channel catfish for harvest weight or dressout percentages; however, triploids had significantly lower survival and yield, and feed conversion efficiency. Macroscopic examination of gonads from diploid and triploid catfish revealed the expected lack of sexual development and gonadal maturation in channel catfish triploids. Triploid catfish gonads were significantly smaller than those of diploid channel catfish. Ovaries from triploid catfish were 3-4 times smaller than diploid channel catfish ovaries.

None of the significant differences I found between polyploid and diploid ictalurids would lead to economic benefit to the catfish industry. Further research with emphasis on tetraploidy and the effects of polyploidy-inducing treatments on different strains of catfish is needed.

INTRODUCTION

Domestic production of channel catfish in 1987 was 127 million kg, with a total value to producers of approximately $179,000,000 (USDA 1988)[1]. The popularity of this species in culture is due to its ease of management and good food quality. Although considerable research has been conducted on culture techniques, less work has been directed toward genetic improvements which could increase its potential for food production.

Triploidy has been successfully induced in many fish species (Swarup 1959, Purdom 1972, Valenti 1975, Gervai et al. 1980, Lincoln 1981, Wolters et al. 1981), and improvements in economically important traits have been demonstrated. Triploid plaice-flounder hybrids, tilapia, and channel catfish are significantly larger than normal diploids (Purdom 1976, Valenti 1975, Wolters et al. 1982). The faster growth rate demonstrated by triploid channel catfish in tank culture would be very beneficial in commercial pond culture. Faster growing fish reach market size faster, resulting in a savings in labor and greater production per unit time. Better feed conversion in triploids, possibly resulting from sterility, yields savings in feed costs which can account for 51-57% of annual operating costs (Waldrop and Smith 1980, Dellenbarger and Vandeveer 1986).

One of the greatest benefits to commercial catfish producers may stem from the increased dressout percentage in triploid catfish. Reduced gonadal development leads to less wastage in processing. Chrisman et al. (1983) reported an average of 6% higher dressout percentage in tank raised triploid catfish.

Whereas triploid catfish are valuable because they are sterile, tetraploid fish could be extremely useful in fish culture because they are to be fertile. Triploid progeny could result from the successful spawning of a tetraploid female with a diploid male. Tetraploidy was successfully induced in rainbow trout (Salmo gairdneri) with heat shocks (Refstie 1981, Thorgaard et al. 1981), and in channel catfish with heat shocks (Bidwell et al. 1985). However, viability and fertility have not been shown and many fish were diploid-tetraploid mosaics. Large numbers of tetraploids need to be induced and evaluated to ascertain the value of tetraploid fish and their feasibility for mass-producing triploids.

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[1]Citation, Table, Figure and Bibliographic styles throughout follow those of the journal Aquaculture.

The principal goals of this study were to induce polyploidy in channel catfish (Ictalurus punctatus) and channel catfish x blue catfish (I. furcatus) hybrids, and evaluate resulting production benefits. Specific objectives of this project were to:

1) induce triploidy and tetraploidy and evaluate the viability and fertility of triploid channel x blue catfish hybrids,

2) compare the growth rate and production characteristics of triploid hybrids with diploid hybrids in tanks from age 14 months to 18 months, and

3) compare the growth rate and production characteristics of diploid and triploid channel catfish in earthen ponds.

STUDY I:
INDUCTION OF POLYPLOIDY IN CHANNEL CATFISH AND ICTALURID HYBRID CATFISH WITH TEMPERATURE AND HYDROSTATIC PRESSURE SHOCKS

ABSTRACT

Lilyestrom, C. G., 1989. Induction of polyploidy in channel catfish and ictalurid hybrid catfish with temperature and hydrostatic pressure shocks. Aquaculture 00:00-00. (In review)

One-cell embryos of ictalurid catfish, including various hybrid combinations, were subjected to either temperature or hydrostatic pressure shocks for induction of triploidy or tetraploidy. Hydrostatic pressure was more consistent than cold-shocks for inducing triploidy with high survival of treated embryos. Pressures of 1300-1500 kg/cm^2 for 5 min at 5 min after fertilization induced 100% triploidy with 50% hatching percentage.

Hatch rates of embryos treated for induction of tetraploidy with hydrostatic pressure shocks were lower than those treated for induction of tetraploidy with heat shocks. In both cases, hatch rates decreased with the increase in treatment level. Heat shock parameters previously reported as being effective for tetraploid induction in channel catfish did not induce tetraploidy in this study.

The most effective heat shock and pressure shock treatments gave similar results, inducing approximately 30-70% tetraploidy. Treatments applied near first cell divisions (80 to 83 min post-fertilization) were more effective for producing tetraploidy than those applied earlier (43 to 75 min post-fertilization) in the cell cycle. Percent tetraploidy was higher when the fish were examined 2 days after fertilization than when examined at 3 months of age suggesting that induced tetraploid catfish are subviable. Diploid-tetraploid mosaics with compressed bodies and skeletal deformities were also produced.

INTRODUCTION

Induction of triploidy is expected to have important economic advantages because of its influence upon the growth and lack of sexual maturation (Valenti 1975; Purdom 1976; Wolters et al. 1982). Triploids grew faster and converted food more efficiently than diploids in some studies (Valenti 1975; Purdom 1976; Wolters et al. 1982; Chourrout and Itskovich 1983). However, triploids sometimes exhibit similar (Swarup 1959) or inferior growth compared to diploids (Gervai et al. 1980; Utter et al. 1983), and the growth benefits of induced triploidy are often not realized until the onset of maturation effects or after the time of maturation in diploids (Chourrout et al. 1986; Myers 1986; Dunham 1988).

Triploidy has been successfully induced with cold-shocks in channel catfish, Ictalurus punctatus (Wolters et al. 1981). Upon reaching 90g, triploid channel catfish grew faster than diploids in tanks (Wolters 1982). Tank-raised triploid channel catfish also have higher dressing percentage than diploids (Chrisman et al. 1983). However, catfish may not perform equally in different production units. Results from tank studies may not be repeatable in pond studies, due to genotype-environment interactions.

Production and performance of hybrid triploid catfish have not been evaluated. Triploid salmonid hybrids have shown improved performance for some culture traits compared to diploid hybrids (Dunham 1988, Seeb et al. 1988, Utter et al. 1983). Tank and pond studies are needed to evaluate possible hybrid triploid catfish benefits.

Induction of tetraploidy has potential advantages. Mechanical induction of triploidy may not always allow production of triploids on a commercial scale. If tetraploids were fertile, they could be mated to normal diploids to naturally produce triploids. Chourrout et al. (1986) were able to produce second generation triploids in rainbow trout at percentages ranging between 94% and 100% by mating tetraploid males with diploid females.

A second potential advantage of inducing tetraploidy could be the retention of fertility in interspecific hybrids by retaining the chromosome set balance. Although channel catfish is the primary culture species among ictalurids, other species have desirable culture traits (Dunham and Smitherman 1984; Plumb 1986; Dunham and Smitherman 1987) that could be introduced to channel catfish through introgression. However, diploid ictalurid hybrids have fertility problems that are probably caused by chromosome incompatibilities and lack of homologue

pairing during meiosis (LeGrande et al. 1984). Induction of tetraploidy and restoration of chromosome set balance might result in fertile allotetraploids capable of producing an F_2 generation that could be used for the selection of recombinant synthetic breeds. If the state of tetraploidy causes reduction of performance as it does in rainbow trout (Chourrout et al. 1986), diploidy could be restored after the mixing of chromosomes by activating the 2n ova of a tetraploid female with irradiated milt (Refstie et al. 1982, Refstie 1983, Chourrout and Nakayama 1987).

Bidwell et al. (1985) produced autotetraploid channel catfish using heat shocks at 40-43 C applied to eggs incubated at 27 C. The tetraploid induction rate was highest, 62%, at 41 C for 3 minutes. However, high percentages of mosaics were also reported. The objective of this study was to evaluate temperature-shocks and hydrostatic pressure-shocks for induction of triploidy and tetraploidy in channel catfish and hybrids with blue catfish, white catfish, and black bullheads.

MATERIALS AND METHODS

12 channel catfish females were paired in April-May 1986 with channel or blue catfish males at the Louisiana Agricultural Experiment Station, Ben Hur Research Facility (LAES). Channel catfish (I. punctatus), blue catfish, (I. furcatus), white catfish, (I. catus), and black bullhead catfish, (I. melas) were also paired (Rezk 1988) in various combinations in May 1986 at the Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station (AAES). The female parent was usually the channel catfish.

Eggs from each female were divided into aliquots of approximately 50-100 per treatment, depending on the number of ovulated eggs available. Control eggs from each mating were raised at 26-27 C at LAES. 10 minutes after fertilization at 26-27 C, embryos were cold-shocked at 5 C for 30 to 60 minutes for induction of triploidy at LAES (Table I). 5 minutes after fertilization at 26-27 C embryos were subjected to hydrostatic pressures of 900-1500 kg/cm^2 for 5 minutes for induction of triploidy at AAES (Table I).

Prior to first cell division, eggs incubated at 26-27 C were exposed to different levels of either temperature or hydrostatic pressure shocks (Dasgupta 1962) of different durations to induce tetraploidy. Temperature-shock treatments ranged from 38 to 42 C for durations of 3 to 6 minutes or 5 C for 45 minutes,

Table I. Ploidy level and percent hatch for ictalurid catfish eggs subjected to cold shocks or pressure shocks for induction of triploidy

Treatment[a]	Mating[b]	N[c]	% 2N	%3N	% Mosaic	% Hatch
Control[d]	CxC	653				40
Control[d]	CxB	219				33
5/30/10[d]	CxB	1	0	0	100	15
5/45/10[d]	CxB	12	84	8	8	25
5/30/10[d]	CxC	6	25	75	0	17
5/45/10[d]	CxC	4	0	100	0	37
5/60/10[d]	CxB	5	0	0	100	5
900/5/5[e]	CxB	19	33	67	0	100
1300/5/5[e]	CxC	11	0	100	0	52
1500/5/5[e]	CxC	31	0	100	0	50

[a] 5 C or 900-1500 kg/cm^2/duration (min)/time after fertilization (min),

[b] C = Ictalurus punctatus, B = Ictalurus furcatus, female x male,

[c] N = number analyzed for ploidy; controls assumed 2n,

[d] Location = Louisiana State University, Ben Hur Aquaculture Research Facility and

[e] Location = Auburn Fisheries Research Unit, Auburn University.

while hydrostatic pressure treatments ranged from 700 to 1500 kg/cm^2 for durations of 4 to 10 minutes (Tables II and III). Shocks were initiated 43 to 90 minutes after fertilization. After the treatment, the embryos were immediately placed at the original incubation temperature. First cleavage occurred approximately 80-90 minutes after fertilization.

After treatments, the eggs were incubated in plastic screen cages approximately 20 x 10 x 15 cm hanging in aerated flowing pond water at 26-27 C. Fry were reared in the window screen cages in which they were hatched. Fry were kept indoors in the cages for 3 weeks, counted, and then moved to 0.04 ha ponds at AAES (Rezk 1988). Fry were reared in hatchery tanks at LAES. When the fish were 3 months old, they were harvested, weighed to nearest gram, and analyzed for ploidy level. A sample (the number depending on the survival percentage) of 2-day-old embryos from each treatment was karyotyped to determine ploidy level. At LAES the karyotyping technique was modified from Hollenbeck and Chrisman (1981). The technique utilized at AAES was modified (Rezk 1988) from the one described by Kligerman and Bloom (1977). A total of 7 to 10 metaphase spreads was examined per slide and 1 to 5 slides were examined per replicate at 1000X.

Erythrocyte nucleus size was measured in 3-month-old fish by the sensing zone technique (Harvey 1968; Benfey et al. 1984) at AAES. A model ZM Coulter Counter (Coulter Electronics) and a model 256 Channelizer (Coulter Electronics) were used to determine the ploidy level in live specimens at AAES. A one-way analysis of variance was used to analyze percentage survival in heat shock treatments.

RESULTS AND DISCUSSION

Induction of Triploidy

Cold-shocks were variable for inducing triploidy (Table I). Channel catfish embryos treated at 5 C for 45 minutes 10 minutes after fertilization were all triploids. Percent hatch for this treatment was 37%, highest among all cold shock treatments. At LAES, percent hatch for embryos cold shocked at 10 minutes after fertilization was not significantly different from the percent hatch of the control embryos for either the channel catfish ($p = .3575$) or the channel female x blue catfish hybrid treatments ($p = .1454$). Previously, shocks 5 minutes after

Table II. Survival of ictalurid catfish at three stages of development after treatment with thermal shocks for induction of tetraploidy

Treatment[a]	Mating[b]	Trials	Survival (%)[d] 6 days	3 weeks	3 months
36/3/80	CxB[f]	1	80		
38/3/80	CxB	1	84		
	CxW	1	78	1	66
	WxB	1	5	12	
	CxB[c]	1	5	12	
39/3/80	CxB	2	74	29	
	CxW	1	67	12	
	WxB	1	67	8	
39/4/75	Cx(B+M)	1	50	10	100
39/4/80	CxB	2	53	16	75
	Cx(B+W)	1	44	30	23
39/5/75	Cx(B+M)	1	1		
39/5/80	Cx(B+W)	1	22	70	77
39/6/75	Cx(B+M)	1	1		
39/6/80	CxB	2	0		
	Cx(B+C)	1	0	50	100
39/6/85	CxB	1	0		
	WxC	1	0		
39/6/90	WxC	1	0		
40/3/75	Cx(B+M)	1	0		
40/3/80	CxB	1	0.5	0	0
40/4/80	CxB	1	0		
	Cx(B+W)	1	0		
40/6/80	CxB	1	0		
	Cx(B+W)	1	0		
41/3/80	CxB	4	0		
	Cx(B+W)	1	0		
	CxC[f]	1	7		
41/3/85	CxB	1	0		
	WxC	1	0		
41/3/90	WxC	1	0		
41/4/80	Cx(B+C)	1	0		
42/3/80	CxB	1	0		

[a] °C/duration of the treatment (min)/time after fertilization (min),

[b] C = Ictalurus punctatus, B = I. furcatus, W = I. catus, M = I. melas, female x male,

[c] Treatment was fluctuating between 38 and 40°C,

[d] 6 day = % hatch relative to control, 3 weeks = survival from hatch to 3 weeks relative to hatch, 3 months = survival from 3 weeks to 3 months relative to survival at 3 weeks,

[e] Hybrids were pooled for this stage, and

[f] Location = L SU, Baton Rouge; all others are Auburn Fisheries Research Unit.

Table III. Percentage of tetraploids at 2 days and at 3 months of age for ictalurid catfish treated with thermal shocks

		2 days			3 months		
Treatment[a]	Mating[b]	N[c]	% Mosaic	% 4N	N	% Mosaic	% 4N
36/3/80	CxB[e]	17	59	6			
38/3/80	CxB	5	0	40	150[f]	0	0
	CxW	5	0	0			
	WxB	5	0	0			
	CxB[d]	3	0	67			
39/3/80	CxB	5	0	0	80[f]	0	0
	CxW	5	0	40			
	WxB	1	0	0			
39/4/80	CxB	6	0	33	30	7	0
	Cx(B+W)	6	0	50	7	14	0
39/5/80	Cx(B+W)	5	0	0	27	0	0
39/6/80	Cx(B+W)	2	0	50			
5/45/80	CxB[e]	3	0	0			
41/3/80	CxC[e]	17	0	0			

[a] °C/duration of the treatment (min)/time after fertilization (min),

[b] C = Ictalurus punctatus, B = I. furcatus, W = I. catus, M = I. melas,

[c] N = number analyzed for ploidy,

[d] Treatment was fluctuating between 38 and 40 C,

[e] Location = Louisiana State University, Baton Rouge; all others are Auburn Fisheries Research Unit, and

[f] All hybrids were pooled.

fertilization for 60 min resulted in highly variable survival rates (Wolters et al. 1981). The same treatment upon channel female by blue male hybrid embryos also had relatively high hatch, 25%, compared to other treatment durations, but did not induce triploidy (Table I). The only hybrid embryo examined which had been cold shocked for 30 minutes, was a mosaic. Survival for this group was only 15%. 30-minute cold shocks produced 75% triploid channel catfish embryos. A cold-shock treatment of 60 minutes for channel catfish resulted in the lowest hatch, 5%, and 100% mosaicism. The success of these treatments was not as high as those previously reported (Wolters et al. 1981).

Hydrostatic pressure gave more consistent results than cold-shock for triploid induction and hatching rate (Table I). Treatments for 5 minutes after fertilization at 900, 1300 and 1500 kg/cm^2 had 67, 100 and 100% triploid induction rates and 100, 52, and 50% hatching rates, respectively at AAES. These results point to the use of hydrostatic pressure as the method of choice for producing triploid catfish.

Induction of Tetraploidy with Heat Shocks

Mean hatch of the heat shocked embryos decreased with an increase in temperature and duration of heat-shock (Table II). Significant differences were found among the treatment levels ($P < 0.01$) and treatment durations ($P < 0.02$). No significant differences were found among the times after fertilization ($P > 0.05$). Mean hatch of embryos treated at 38 C for 3 min and 39 C for 3 min was 76% and 71%, respectively, relative to untreated controls. Mean hatch of embryos treated at 39 C for 4, 5, and 6 min was 50, 13, and 0.3%, respectively. Mean hatch for embryos treated at 40 C for 3 min was 0.3%. All embryos treated at 40 C for longer durations, or treated at 41 or 42 C died at AAES, but embryos treated at 41 C for 3 min had 7% hatch at LAES.

These results are in contrast to those of Bidwell et al. (1985). They found hatching rates similar to controls for the embryos treated at 40 and 41 C. Hatch rates were also high (40-78%) at 42 C for 1 minute, and some hatch was also obtained at 42 C for 2 min (0-11%) and 43 C for 1 min (0-73%). Bidwell et al. (1985) found hatchability of fish subjected to 4 min treatment durations to be significantly lower than from shorter shocks (1, 2 or 3 min). Apparently, unknown factors, genetic or environmental, including perhaps catfish strain

differences and water chemistry, make certain ploidy level manipulations difficult to duplicate from one laboratory to another.

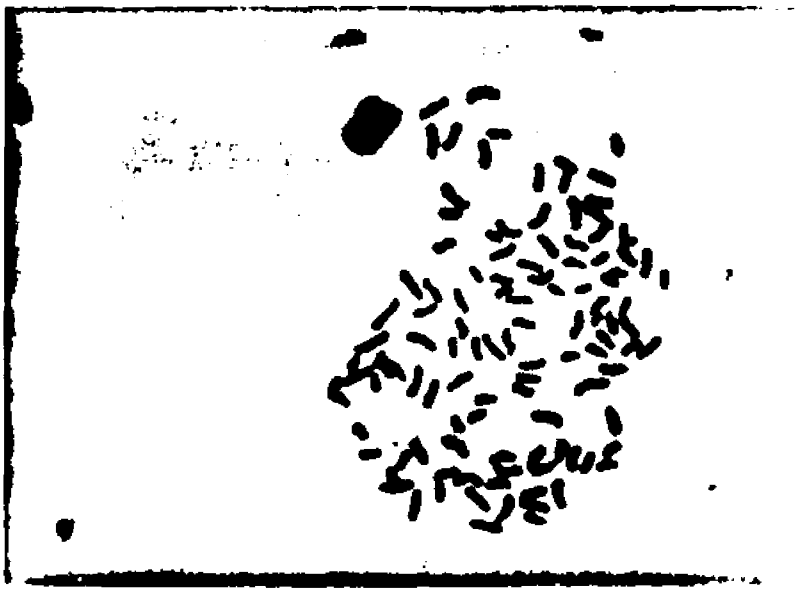
Survival of fry reared indoors from hatch to 3 weeks of age also decreased with increasing shock temperature. Mean survival of fry treated at 38 C for 3 min and 39 C for 3 min was 41% and 21% , respectively (Table II). Mean survival of fry treated at 39 C for 4, 5, and 6 min was 18%, 35%, and 12.5%, respectively. The only individual that hatched from the 40 C shock died at AAES. Apparently, the effects of heat shocks continued into this stage of development.

Survival of fry stocked in ponds and reared from 3 weeks of age to 3 months of age ranged from 35 to 100% and averaged 66% for all heat shock treatments (Table II). These values are typical for channel catfish during this culture phase (Jensen et al. 1983). Observed survival of fry from the most severe treatments was as high or higher than that of fry from the lower temperature shocks or the shorter duration treatments. Apparently, there were no long term (3 months) effects of the heat shocks on survival after the advanced fry stage was reached.

With the exception of the 39 C for 5 min, and 41 C for 3 min, tetraploidy (Fig. I) occurred at all the temperature levels and treatment durations applied. In 2-day-old embryos, the 36 C for 3 min treatment produced 6% tetraploids and 59% diplo-tetraploid mosaics (Table III). The 38 C for 3 min and 39 C for 3 min treatments induced tetraploidy levels of 13% and 18%, respectively. With the exception of 39 C for 5 min, tetraploid induction increased with the increase in the shock duration at AAES. Tetraploid induction rates were 42%, 0%, and 50% at 39 C for 4, 5, and 6 min, respectively. The 41 C for 3 min treatment at LAES did not produce tetraploids.

These percentages are in agreement with those obtained by Bidwell et al. (1985) at higher temperatures, with the exception of the lack of tetraploids among fry treated at 41 C for 3 min. Their best tetraploid percentage (62%) was obtained at 41 C for 3 min. Bidwell et al. (1985) also reported a high percentage of mosaics (approximately 25%) with different combinations of 2n, 3n, 4n, and 5n cells within the same specimen.

The tetraploid percentage in the population decreased as the fish became older (Table IV). The only tetraploids obtained at 3 months of age were among fry treated at 39 C for 4 min where the percentage decreased from 42% to 6%. Differential mortality of tetraploids compared to diploids could be a possible explanation.



Scale

_____ = 0.1 mm

Figure I. Metaphase plate from a kidney cell of a tetraploid channel (Ictalurus punctatus) x blue (I. furcatus) catfish hybrid (4n = 116).

Table IV. Survival of ictalurid catfish at three stages of development after treatment with hydrostatic pressure shocks for induction of tetraploidy

Treatment[a]	Mating[b]	Trials	Survival (%)[c]		
			6 days	3 weeks	3 months
700/5/85	CxB	1	0	0	0
	WxC	1	50	100	7
700/10/75	Cx(B+M)	1	25	8	100
900/4/75	Cx(B+M)	1	25	36	78
900/4/80	CxB	3	0		
	WxB	1	33	33	12
	CxW	1	22		
	CxB[d]	1	7		
	CxC[d]	1	30		
900/4/90	CxB[d]	1	5		
	CxC[d]	1	23		
900/4.5/80	Cx(B+C)	1	0		
900/10/75	Cx(B+M)	1	6	25	100
1200/5/83	WxC	2	5		
1300/5/43	CxC	2	20		
1300/5/67	CxC	2	34		
1300/5/83	CxC	2	14		
1500/5/43	CxC	2	21		
1500/5/67	CxC	2	8		
1500/5/83	CxC	2	1		

[a] kg/cm^2/duration of the treatment (min)/time after fertilization (min),

[b] C = Ictalurus punctatus, B = I. furcatus, W = I. catus, M = I. melas,

[c] 6 day = percentage hatch relative to control, 3 weeks = survival from hatch to 3 weeks, 3 months = survival from 3 weeks to 3 months, and

[d] Location = Louisiana State University, Baton Rouge; all others are Auburn Fisheries Research Unit.

Reduced vitality of induced polyploids has been observed in other fishes. Thorgaard et al. (1982) considered differential mortality as a possible explanation for differences in the percentage of triploidy at different ages in the same group of rainbow trout treated with heat shocks for the induction of triploidy. They also observed the same phenomenon in tetraploids at 18 months of age. Survival of tetraploid rainbow trout was less than that of triploids and diploids (Chourrout et al. 1986). Allotetraploid and autotetraploid tilapia were detected prior to hatch, but none were detected by the end of the sac-fry stage (Myers 1986).
A total of 3 tetraploids survived to the 3 months. They had compressed bodies and caudal deformities. Their pattern of erythrocyte nuclei volume distribution indicated that they could be mosaics. Bidwell et al. (1985) did not observe any abnormal swim-up fry resulting from heat shock treatments that induced tetraploidy. This was in contrast to tetraploid rainbow trout embryos which had a high frequency of abnormalities (Thorgaard et al. 1981; Chourrout 1982, 1984).

Induction of Tetraploidy with Hydrostatic Pressure

Percentage hatch of pressure shock treated embryos was low compared to controls (Table III). Hatch of embryos at AAES treated at 700 kg/cm^2 for 5 and 10 min, and 900 kg/cm^2 for 4 and 10 min was 25, 25, 13, and 6% respectively, relative to the untreated diploid controls. 900 kg/cm^2 treatments had 33% hatch at LAES. Various treatments at 6500 to 1500 kg/cm^2 had hatch rates between 1 and 34%. Hatch decreased as the timing of the treatment approached first cell division (Table III). The observed percent survival also decreased as the treatment level and duration increased. Decreased survival with increase in treatment duration was also reported in rainbow trout subjected to hydrostatic pressure shock treatments that induced tetraploidy (Chourrout 1984).

Survival of fry reared indoors and treated at 700 kg/cm^2 for 5 and 10 min, and 900 kg/cm^2 for 4 and 10 min was 100%, 8%, 11.5% and 25%, respectively. The low survival of fry in most of the treatments indicates that there were long term (3 weeks) effects of the treatments on survival.

Fry reared in ponds at AAES from 3 weeks of age until 3 months of age that were treated at 700 kg/cm^2 for 5 and 10 min, and 900 kg/cm^2 for 4 and 10 min. Survival was 7, 100, 40, and 100% respectively, and averaged 62%. These survival rates are normal for channel catfish at this culture stage (Jensen et al. 1983).

Mean percent tetraploidy in 2-day-old embryos was 58% at 900 kg/cm^2 80 min after fertilization at AAES, and 13% tetraploidy with 58% mosaicism at LAES (Table V). This percentage was similar to the best percentage of tetraploidy in the heat shock treatments. Treatments at 6500 to 1500 kg/cm^2 resulted in as much as 33% tetraploidy. Treatments 80 to 83 min after fertilization produced higher percentage tetraploidy than those at 43, 67, 75 and 90 min after fertilization indicating the time near 80 to 83 min may be critical for tetraploid induction in ictalurid catfish. Tetraploidy was induced in a longer range of times of the first cell cycle in Morone bass (Curtis et al. 1987). Percent tetraploidy was reduced when 3-month-old fingerlings were examined (Table V). No tetraploidy was observed in the 700 kg/cm^2 treatments. Mean percent tetraploidy in the 900 kg/cm^2 treatments was reduced from 58% to 16%. Again, differential mortality could be a possible explanation for this reduction in tetraploid percentage.

Several pond-reared individuals suspected to include tetraploids died prior to analysis despite extreme care in harvesting and handling. Hybrid catfish are usually hardy and tolerant to relatively rough handling (Chappell 1979; Smitherman and Dunham 1985) suggesting that the catfish may have suffered from treatment side-effects.

Temperature shock and hydrostatic pressure both induced polyploidy in ictalurid catfish. Hydrostatic pressure was more consistent than cold-shocks for inducing triploidy. Pressures of 1300-1500 kg/cm^2 for 5 min at 5 min after fertilization induced 100% triploidy with 50% hatching rate. Heat shock and hydrostatic pressure both induced tetraploidy. Tetraploidy was not observed in fish subjected to heat shocks previously reported as effective for tetraploid induction in channel catfish. Treatments at 80-83 min after fertilization were the most effective for producing tetraploidy at 38-39 C. Tetraploids were subviable. Mosaics were also produced and they had compressed bodies and caudal deformities.

The growth and survival of the triploid and tetraploid ictalurids should be evaluated. The tetraploids should be grown to maturity, their fertility and spawning behavior determined, the nature of their meiotic division studied, and the performance of their progeny determined to establish the feasibility of using tetraploids for natural production of triploids or for combining the genomes of ictalurid catfishes through introgression.

Table V. Percentage of tetraploidy at 2 days and at 3 months of age for ictalurid catfish treated with hydrostatic pressure shocks

		2 days			3 months		
Treatment[a]	Mating[b]	N[c]	% Mosaic	% 4N	N	% Mosaic	% 4N
4000/5/85	WxC				7	0	0
4000/10/75	Cx(B+M)				1	0	0
900/4/75	Cx(B+M)	1	0	0	14	0	14
900/4/80	WxB	3	0	66	3	0	33
900/4/80[d]	CxB	3	66	0			
900/4/80[d]	CxC	4	50	25			
900/4/90[d]	CxB	3	33	0			
900/4/90[d]	CxC	4	0	0			
900/4.5/80	WxB	4	0	50			
900/10/75	WxC	2	0	0			
6500/5/83	Cx(B+W)	27	0	7			
1300/5/43	CxC	8	0	0			
1300/5/67	CxC	8	0	0			
1300/5/83	CxC	11	0	27			
1500/5/43	CxC	14	0	0			
1500/5/67	CxC	6	0	17			
1500/5/83	CxC	6	0	33			

[a] psi/duration of the treatment (min)/time after fertilization (min),

[b] C = Ictalurus punctatus, B = I. furcatus, W = I. catus, M = I. melas,

[c] N = number analyzed for ploidy, and

[d] Location = Louisiana State University, Baton Rouge; all others are Auburn Fisheries Research Unit.

STUDY II:

COMPARATIVE PERFORMANCE OF DIPLOID AND TRIPLOID CHANNEL X BLUE CATFISH HYBRIDS

ABSTRACT

Lilyestrom, C.G., 1989. Comparative performance of diploid and triploid channel x blue catfish hybrids. Aquaculture 00:00-00. (In preparation).

Allotriploidy (3n = 87) was successfully induced in channel catfish x blue catfish hybrids by cold shocking eggs at 10 min post-fertilization for 30-60 min at 5 C. It was hypothesized that triploid hybrids may have the advantages of heterosis as well as sterility caused by triploidy. No significant differences (α = .05) were detected in growth, feed efficiency, gonadosomatic index or dressout characteristics between diploid and triploid hybrids raised in 1.3-m diameter tanks, with the exception of the significantly higher (P = 0.0321) condition factor (K) of triploid hybrids. All diploid hybrids had normal gonadal development and histology; however, triploid hybrids had abnormal gonadal development with a single ovary present in 36% of triploid females. Both testes were present in all males; however, triploid hybrid males had abnormal histology with no sperm present in seminiferous tubules. Sex ratios did not differ significantly from the expected 1 : 1 ratio in fish from either treatment. Survival for both treatment groups was 100% from age 14 months to 18 months.

INTRODUCTION

Domestic production of channel catfish in 1987 was 127 million kg, with a total value to the producers of approximately $179,000,000 (USDA 1988). The popularity of the species in culture is due to its ease of management and good food quality. Although considerable research has been conducted on culture techniques, less work has been directed toward genetic improvements which could increase its potential for food production.

Induced polyploidy is a potential method for genetic improvement in many species because of the immediate benefits it may provide (Thorgaard 1986). Induced polyploidy is a genetic tool that could significantly change economically important traits in an organism, such as sterility, increased growth rate, and enhanced dressout percentage in a single generation. Genetic selection, on the other hand, may require several generations before a comparable gain can be realized.

Triploidy has been successfully induced in many fish species (Swarup 1959, Purdom 1972, Valenti 1975, Gervai et al. 1980, Lincoln 1981, Thorgaard et al.1981, Wolters et al. 1981, Ueno and Arimoto 1982, Cassani and Caton 1986, Krasnai and Marian 1986), and improvements in economically important traits have been demonstrated. Triploid plaice-flounder hybrids, tilapia, and channel catfish are significantly larger than normal diploids (Purdom 1976, Valenti 1975, Wolters et al. 1982). Triploidy has proven useful for increasing survival in salmonid hybrids (Scheerer and Thorgaard 1987, Seeb et al. 1988). The faster growth rate demonstrated by triploid channel catfish in tank culture would be very beneficial in commercial pond culture (Chrisman et al. 1983). Faster growing fish reach market size faster, resulting in a savings in labor and greater production per unit of time. Better feed conversion in triploids, possibly resulting from sterility, yields savings in feed costs which can account for 51-57% of annual operating costs and 42-47.5% of total annual costs, which include capital costs and taxes (Dellenbarger and Vandeveer 1986). One of the greatest benefits to commercial catfish producers may stem from the increased dressout percentage in triploid catfish. Reduced gonadal development leads to less wastage in processing. Chrisman et al. (1983) reported an average of 6% higher dressout in tank raised triploid channel catfish.

Hybridization has also been successful between several catfish species. Hybrid channel x blue catfish (2n = 58) are more uniform (Brooks 1977), grow approximately 18% faster (Yant et al. 1975, Chappel 1979, Tave et al. 1981), and have a greater dressing percentage (Yant et al. 1981) than channel catfish. Channel x blue catfish

hybrids are fertile (LeGrande et al. 1984), and it was thus hypothesized for this study that sterile triploid hybrids might have the advantages of heterosis as well as triploidy.

MATERIALS AND METHODS

12 sexually mature male and female channel catfish were paired in spawning tanks in May 1986. Daily injections of human chorionic gonadotropin (HCG) at 1,100-1,800 IU/kg were used to induce ovulation in female channel catfish. When a female was ovulating freely, she was anesthetized with tricaine methane sulfonate (MS-222) at 150 ppm, eggs were hand-stripped, fertilized in an 0.65% NaCl solution with sperm from mascerated testes of blue catfish males, and cold shocked at 5 C beginning 10 minutes after fertilization for 30, 45, or 60 minutes duration to produce triploid hybrid catfish.

Following the cold shock, eggs were transferred without acclimation to a plastic-screen basket in a standard hatching trough with well-aerated water at 24-28 C. Success of triploid induction was determined in fingerlings by chromosome counts of dividing cells from mascerated kidney tissue (Hollenbeck and Chrisman 1981). Randomly selected diploid and triploid hybrid fingerlings were stocked in 1.3-m diameter fiberglass tanks (covered with 1 cm mesh netting) at a rate of 8 per tank with 3 replicate tanks for each ploidy level. Tanks were equipped with upflow biological filters with a recirculation rate of approximately 15 l/min. Differential fry rearing densities, resulting from early life stage mortality, resulted in stocking length and weight differences between the treatment groups. Diploids were significantly larger than triploids when stocked ($P < 0.05$) (Table I).

Water temperature and dissolved oxygen were measured daily using a Yellow Springs Instruments oxygen/temperature meter. Partial water exchanges with ambient pond water (approximately 1/3 of tank volume) were performed weekly.

Fish were fed floating catfish feed (32% protein) daily as a percentage of their body weight (DuPree and Huner 1984), varying from 1 - 3% per day. Fish were not fed on length/weight sampling days. Biomass estimates were updated monthly following the length/weight measurements. Daily feed amounts were recorded for each tank for calculations of feed efficiency. Feed efficiency was calculated from the grams of feed consumed divided by the grams of fish produced (Swingle 1959).
Growth, as measured by weight (to the nearest 1 g) and total length (mm), were recorded monthly for all fish. During the data collection procedure, fish were anesthetized with MS-222 at approximately 150 ppm. Dressout percentage ((headed,

Table I. Mean (± S.D.) weights and condition factor (K) of diploid and triploid hybrid catfish from 14 months to 19 months of age

	Diploids			Triploids		
Age (Months)	Mean weight (g ± S.D.)	K[1]	n[2]	Mean weight (g ± S.D.)	K	n
14	33.5±16.6	7.0±1.0	24	20.5±8.1	7.3±1.4	24
15	65.5±24.6	8.6±0.8	24	65.0±26.8	10.6±2.5	24
16	91.7±31.1	8.5±0.9	24	106.3±46.5	10.8±2.8	24
17	122.2±41.8	9.1±1.6	24	130.9±59.2	10.5±2.4	24
18	150.4±54.4	8.7±1.1	24	154.5±73.4	9.9±2.4	24
19	182.7±81.1	8.6±1.8	16	173.9±84.9	9.6±1.9	24

1 $K = \frac{W}{L^3} X$ where W equals weight (g), L equals length (mm), and X is a scaling constant.

2 n = number of fish.

gutted and skinned weight/total weight) x 100) and the gonadosomatic index were calculated for the diploid hybrid and triploid hybrid catfish after a growout period of 150 days (18 months age).

The following function was used to calculate the specific growth rate (g) (Krasnai and Marian 1986):

$$g = \frac{\ln (W_t - W_O)}{t}$$

where W_t is the weight at time t, and W_O is the initial weight.
Condition factors (K) (Nielsen and Johnson 1983) were calculated as follows:

$$K = \frac{W}{L^3} X$$

where W equals weight (g), L equals length (mm), and X is a scaling constant.
Gonadosomatic index (GSI) was calculated as follows (Kaya and Hasler 1972):

$$GSI = \frac{W}{B} X\ 100$$

where W is the weight (g) of both gonads, and B is the total weight (g) of the fish, including gonads.

Ovaries and testes from all diploid and triploid hybrid catfish were removed and fixed in Bouin's fluid at harvest (18 months age). Tissue samples were dehydrated in an alcohol series and mounted in paraffin. Tissues were sectioned to a thickness of 6 µm and stained with hematoxylin and eosin. Slides were examined and photographed through a Zeiss photomicroscope.

The significance level used in all statistical tests was $\alpha = 0.05$. Chi square was used to test for variation from the expected 1:1 sex ratio. Unpaired, two-way Student's t-tests were used to test for significant differences between most diploid vs. triploid hybrid characteristics. Two-way analyses of variance were used to test for interaction between ploidy and sex. Analyses of covariance were used to compensate for differences in stocking sizes of the treatment groups when testing for differences in growth between the ploidy levels.

RESULTS

Dissolved oxygen levels and water temperature did not differ significantly among the tanks. Temperature and dissolved oxygen means (± S.D.) were 24.1 ± 4.5 C and 7.4 ± 1.1 mg/l, respectively. Ranges (minimum - maximum) for temperature and dissolved oxygen were 12.5 - 34 C and 4.2 - 11.3 mg/l, respectively.

Allotriploidy (3n = 87) was successfully induced in channel x blue catfish hybrids, as determined by chromosome counts from metaphase spreads of kidney tissue (Fig. I). Covariance analysis of the weights of triploid hybrids and diploid hybrids at the sample times indicated that the 2 groups had similar slopes *b* ($P = 0.954$) but different intercepts *a* ($P < 0.01$) due to differences in stocking weights (Table I). The specific growth rate "g" did not differ significantly between the 2 groups ($P = 0.133$) (Table II). Survival for both groups was 100%.

Condition factor (K) for hybrid triploid catfish was significantly different ($P = 0.0321$) from that of the diploid hybrid catfish at 18 months of age. Mean condition factors for diploid and triploid hybrid catfish were 8.7 ± 1.1 and 9.9 ± 2.4, respectively (Table I). K was highest in both treatments at age 16 to 17 months (catfish were stocked in July at age 14 months), declining thereafter possibly as a response to decreasing feeding activity at lower water temperatures in fall and winter months.

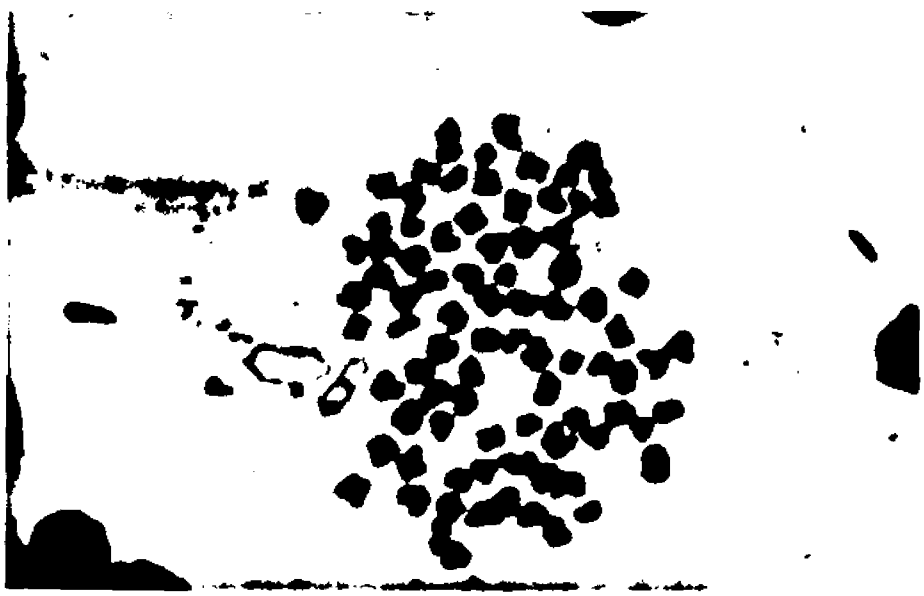
Feed conversion ratios (FCR) varied significantly over time ($P = 0.0249$), but did not differ between the triploids and diploids ($P = 0.2342$). Mean feed conversion ratios for combined sexes of hybrid diploids and hybrid triploids were 2.6 ± 1.5 and 2.1 ± 1.2, respectively (Table II). Chappel (1979) calculated a feed conversion ratio of 1.21 : 1 for channel x blue catfish hybrids raised in earthen ponds. Andrews and Stickney (1972) obtained lowest feed conversions (1.4 : 1) in channel catfish at 30 C and a feeding rate of 2% of body weight. Lower mean water temperature in this study may have also contributed to the higher feed conversion ratios.

Gonadosomatic index (GSI) was not significantly different between diploid and triploid hybrid catfish ($P = 0.785$) (Table II). All diploid hybrids had visibly normal gonadal development, which is consistent with the observations reported by LeGrande et al. (1984). Four out of eleven (36%) triploid hybrid females had a single ovary; both ovaries were present in the remaining seven females. Histology of diploid ovaries showed numerous developing oocytes (Grizzle and Rogers 1976) (Fig. III). Histological examination of triploid hybrid ovaries revealed few normally developing

Table II. Processing percentages, growth parameters, survival (to 18 months), and feed conversion ratios (mean ± S.D.) of diploid and triploid hybrid catfish.

	Diploid Hybrids		Triploid Hybrids	
	Males	Females	Males	Females
Total Wt. (g)	178.7±88.0	169.0±64.9	212.7±101.1	149.4±64.6
Gonad Wt. (g)	0.219±0.1	0.833±0.6	0.242±0.2	0.804±0.6
GSI	0.13±0.1	0.55±0.3	0.12±0.1	0.54±0.3
Fillet Wt. (g)	113.0±56.7	110.0±37.7	131.2±61.3	93.2±43.0
Dressout %	62.8±2.1	68.6±13.9	62.2±4.8	61.5±4.2
Survival %	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
FCR	2.6 ± 1.5		2.1 ± 1.2	
"g"[1]	1.99±1.2		2.14±1.4	

[1] Specific growth rate $g = \frac{\ln (W_t - W_O)}{t}$ where W_t is weight at time t, and W_O is initial weight.



Scale

———————— = 0.1 mm

Figure 1. Metaphase plate from a kidney cell of a triploid channel (Ictalurus punctatus) x blue (I. furcatus) catfish hybrid (3n = 87).

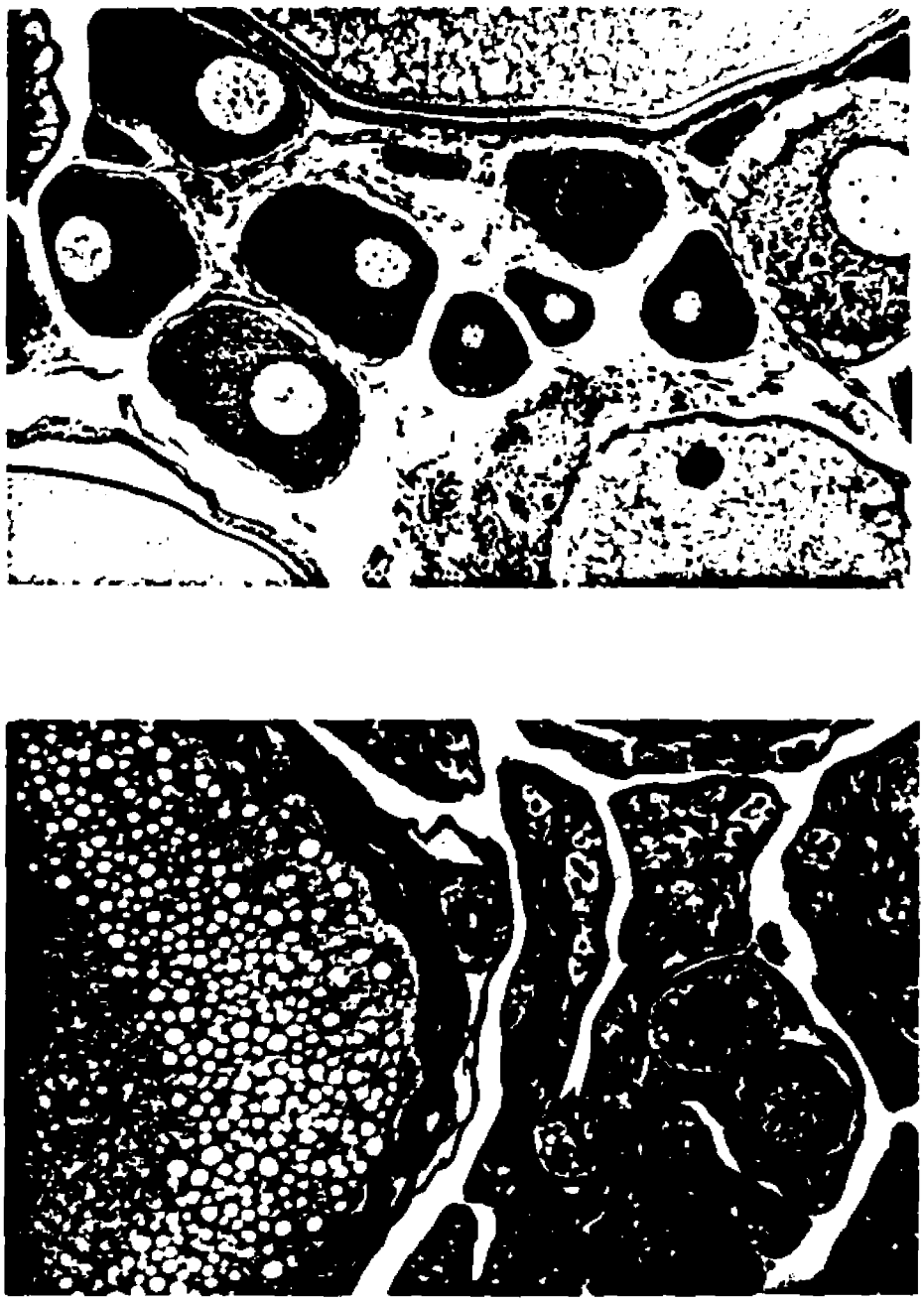


Figure II. Histological sections of ovaries from diploid (above) and triploid (below) channel (Ictalurus punctatus) x blue (I. furcatus) catfish hybrids.

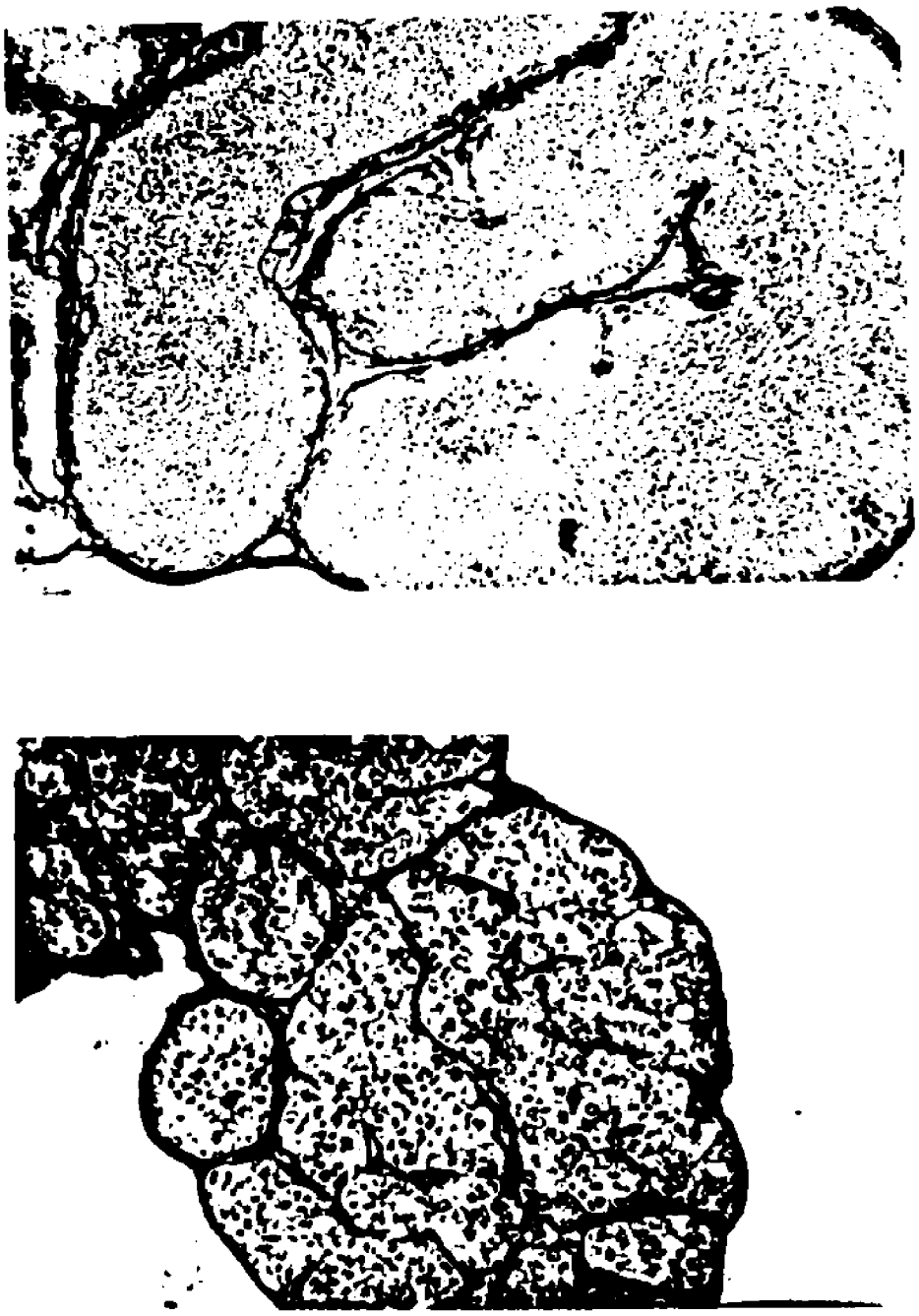


Figure III. Histological sections of testes from diploid (above) and triploid (below) channel (Ictalurus punctatus) x blue (I. furcatus) catfish hybrids.

oocytes (Fig. II) and large numbers of abnormally dividing cells, similar to ovaries of triploid channel catfish (Wolters et al. 1982). Both testes were present in all male diploid and triploid hybrids. Triploid hybrid males had abnormal seminiferous tubules with no sperm present and were similar in appearance to testes of triploid channel catfish (Wolters et al. 1982). One triploid hybrid could not be sexed by gross examination of the gonadal tissue, and was excluded from all analyses separated by sex. Chi-square tests indicated the sex ratio did not differ significantly from the expected 1 : 1 in either diploid hybrids (P = 0.705) or triploid hybrids (P = 0.756).

Dressout percentage did not differ significantly between the fish from diploid and triploid hybrid treatments (P = 0. 3451), nor did it differ between the 2 sexes at each ploidy level (P = 0.393) (Table II). Dressout percentage varied more between the sexes of diploid hybrids (male $\bar{X}$ = 62.9 ± 2.2; female $\bar{X}$ = 65.5 ± 10.7) than between the sexes of triploid hybrids (male $\bar{X}$ = 62.7 ± 6.6; female $\bar{X}$ = 61.5 ± 4.2). Chappel (1979) reported 62.0 % dressout for diploid channel x blue hybrids.

DISCUSSION AND CONCLUSIONS

The first reported triploid fish hybrids (Pleuronectes platessa x Platichthys flesus) were induced via cold shock by Purdom (1972). Ueda et al. (1984) encountered spontaneous digynic triploidy in Salvelinus fontinalis x Salmo gairdneri hybrids. These are the first allotriploids reported in Siluriformes.

Triploid hybrids did not outperform diploid hybrid catfish in growth, feed conversion, or dressout percentage. Significant differences in the condition factor (K) indicated that triploid hybrids were significantly heavier at any given length than diploid hybrids. However, skeletal deformities were observed in several triploids, and therefore the difference might be interpreted as the triploids being shorter, rather than of greater robustness. Johnson et al. (1986) observed no difference in growth parameters of diploid and triploid Coho salmon. In contrast, Benfey and Sutterlin (1984) found triploid Atlantic salmon had a lower condition factor than diploids. Cassani and Caton (1986) found a significantly higher condition factor in diploid grass carp when grown together with triploids. No mention was made of skeletal deformities in any of these studies.

Triploid fish are generally functionally sterile, with underdeveloped gonads (Chourrout 1980, Wolters et al. 1982, Lincoln and Scott 1984, Cassani and Caton 1986, Krasnai and Marian 1986). However, in this study, no significant difference was found in the gonadosomatic index of diploid hybrids vs. triploid hybrids, even

though 4 out of 11 triploid females had a single visible ovary, and histological examination showed gonads of triploids to be underdeveloped. The lack of significance may be due to the immature age (19 months) of the study fish. Gonads were in an early stage of development in all fish at the time of examination.

The potential value of triploid hybrid catfish to the industry from sterility and hybrid vigor was not found in this study. In the present study, the desirable production characteristics, faster growth, better feed conversion, and higher dressout percentage, which had been found in triploid channel catfish (Wolters et al. 1982, Chrisman et al. 1983), were not detected in channel catfish x blue catfish triploid hybrids. At this time, first generation triploids and hybrid triploids still remain difficult to produce in commercial quantities, and are unpredictable in their performance.

STUDY III:
POND GROWTH, YIELD, AND DRESSOUT PERCENTAGES OF DIPLOID AND TRIPLOID CATFISH, ICTALURUS PUNCTATUS

ABSTRACT

Lilyestrom, C.G., 1989. Pond growth, yield, and dressout percentage of diploid and triploid channel catfish, Ictalurus punctatus. (In preparation).

Fingerling diploid and triploid channel catfish, 63 ± 14 and 45 ± 17g, respectively, were stocked in 0.04 ha earthen ponds at 11,000 fish/ha with three replicate ponds at each ploidy level. Fish were fed a pelleted commercial catfish ration (32% protein) to satiation once daily. Total weight and survival were recorded from each pond after 185 days. No significant difference was found between diploid and triploid catfish for harvest weight and dressout percentage; however, triploids had significantly ($p < 0.05$) lower survival, yield, and higher feed conversion. Macroscopic examination of gonads revealed the expected lack of sexual development and gonadal maturation in triploids.

INTRODUCTION

Channel catfish are widely cultured because of high flesh quality and ease of culture. Considerable research has been performed on rearing, stocking, and nutrition of channel catfish, but less research has been directed toward genetic improvements of production characteristics (Tucker 1985).

Growth potential of triploid fish has been compared with that of diploids in several species (Cassani and Caton 1986, Krasnai and Marian 1986, Johnson et al. 1986). Growth of triploids has generally been significantly better than diploids during the sexual maturation phase. Limited experimental research on triploid channel catfish raised in indoor tanks (Wolters et al. 1982; Chrisman et al. 1983) demonstrated that induced triploidy can increase production in cultured channel catfish through increased growth rates, feed conversion efficiency, and processing characteristics. However, triploid channel catfish have never been evaluated in pond culture situations to accurately assess their value in commercial culture. Results of the first evaluation of triploid channel catfish grown in earthen ponds are reported in this study.

MATERIALS AND METHODS

8 sexually mature male and female channel catfish (1.4 - 3.0 kg) were paired in spawning tanks. Daily injections of human chorionic gonadotropin (HCG) at 1,100-1,800 IU/kg were used to induce ovulation in female channel catfish. When a female was ovulating freely, she was anesthetized with tricaine methane sulfonate (MS-222) at 150 ppm. Eggs were hand-stripped, fertilized with sperm from mascerated testes of channel catfish males (Dupree et al. 1969), and cold shocked at 5 C for 60 minutes, beginning 5 minutes after fertilization (Wolters et al. 1981). Following the cold shock, eggs were transferred without acclimation to screen-lined hatching baskets in a standard hatching trough with pond well water at 24-28°C. Success of triploid (3n = 87) induction was determined prior to stocking by chromosome counts of dividing cells from mascerated kidney tissue from 30 fingerlings (Hollenbeck and Chrisman 1981).

Fingerling diploid (63 ± 14 g) and triploid (45 ± 17 g) channel catfish were stocked into 0.04 ha earthen ponds at a rate of 11,000 fish/ha with three replicate ponds at each ploidy level. Fish were fed a commercial floating feed (32% protein) to satiation once daily.

After a growout period of 185 days, all fish were harvested, counted, and weighed to the nearest 1 g. Gonads were removed and weighed to the nearest 0.1 g. The gonadosomatic index (GSI) was calculated as follows (Kaya and Hasler 1972):

$$GSI = \frac{W}{B} \times 100$$

where W is the weight (g) of both gonads, and B is the total weight (g) of the fish, including gonads. Dressout percentage was calculated on 100 fish from each ploidy level from both headed, eviscerated and skinned fish and filleted fish. Whole fish were headed with a band saw, eviscerated by vacuum, and skinned with a Townsend skinner. Individual sex could not be determined on headed, eviscerated and skinned fish because the internal organs were removed by vacuum. Shank fillets (without belly meat) were removed from whole fish and skinned on a Townsend skinner. Fillet dressout percentages were calculated on each sex separately. One-way analyses of variance were conducted on survival, harvest weight, yield, feed conversion, GSI and dressout percentage.

RESULTS AND DISCUSSION

Total weight of diploid and triploid channel catfish did not differ significantly at harvest ($p = 0.5959$). Mean weights for diploid and triploid catfish were 0.52 kg ± .03 (SE) and 0.54 kg ± .07 (SE), respectively (Table I) which constitute acceptable commercial harvest sizes (Dupree and Huner 1984). Yield and survival of triploid channel catfish were significantly lower ($P = 0.0245$ and $P = 0.0435$, respectively) than diploids. Although harvest weights were not significantly different, the lower pond yield in triploids is a direct result of the lower survival.

Results of this study are an apparent contrast to those of earlier studies comparing growth, survival and processing characteristics of triploid and diploid channel catfish grown in indoor tanks. Wolters et al. (1982) found triploid catfish raised in indoor tanks to be significantly heavier than diploids after age 8 months possibly resulting from better feed conversion. Feed efficiencies in this study, however, were significantly higher for triploids ($P = 0.0436$). Feed conversion ratios for pond-raised diploid and triploid channel catfish were 1.17 ± .15 and 1.37 ± .17, respectively (Table I), as compared to the 1.30 and 1.19 calculated for tank-raised diploid and triploid channel catfish, respectively (Wolters et al. 1982). The low survival rate of pond-raised triploids may be partly responsible for the higher feed conversion ratio. Differences in the strains of catfish used could also influence the between-studies comparisons.

Several other studies comparing growth of diploid and triploid fish have indicated that growth of triploids may be only equal to or even inferior to that of

Table I. Mean (±SE) harvest weight, yield, survival, feed conversion and processing percentages of diploid and triploid channel catfish grown in outdoor ponds.

	Diploids		Triploids	
Harvest Weight (Kg)	0.52 ± .03		0.54 ± .07	
Yield (Kg/Ha)	4,188 ± 565		3,012 ± 376*	
Survival (%)	89 ± 8.1		62 ± 8.7*	
Feed Conversion Ratio	1.17 ± .15		1.37 ± .17*	
Headed, gutted, skinned dressout %	56.9 ± 6.3		55.5 ± 3.2	
	Female	Male	Female	Male
Gonad Wt.(g)	7.8 ± 13.64	1.1 ± 0.67	1.8±3.1*	1.5±.49
Gonadosomatic index	1.6 ± 3.0	0.19 ± .15	0.71 ± .74*	0.17 ± .09
Fillets dressout %	28.8 ± 3.6	27.9 ± 3.6	28.5 ± 1.6	27.4 ± 1.7

* $P < 0.05$.

diploids. Swarup (1959) found no difference between growth of diploid and triploid threespine sticklebacks. Gervai et al. (1980) reported that growth of triploid juvenile common carp was not significantly different from that of diploids. Benfey and Sutterlin (1984) found no significant difference in weight of diploid and triploid Atlantic salmon, but triploid fish had a lower condition factor. Johnson et al. (1986) observed no difference in growth parameters of diploid and triploid Coho salmon. Cassani and Caton (1986) found that whenever diploid and triploid grass carp were grown together, diploid fish grew faster and had a significantly higher condition factor than triploids.

The effect of reduced gonadal development was evident in the difference between diploid and triploid gonad weights and gonadosomatic indices. Gonads (both sexes combined) of triploid catfish were significantly smaller ($P = 0.0352$) than those of diploids. This is especially evident when comparing the GSI of female diploid and triploid catfish (1.6 ± 3.0 and $.71 \pm .74$ respectively). Wolters et al. (1982) also found ovaries from diploids to be 3-4 times larger than ovaries from triploids.

There was no significant difference in dressout percentages between triploid and diploid catfish (Table I). Although significant differences were found in gonadal weights between diploid and triploid pond-grown catfish, the gonad weight difference between ploidy levels (approximately 6 g in females) may not result in a significant difference in dressout percentage. Dressout percentages for headed, eviscerated, and skinned fish were slightly lower than reported values from previous studies. This method usually gives a processing yield of approximately 60-65% for headed, eviscerated and skinned fish and 40-45% for filleted fish (Tucker 1986; Ghavimi et al. 1987). However, Russell (1972) reported yields 55 to 60% of live weight, which includes the range found in this study for headed, gutted and skinned fish. The shank fillet percentages (not including belly flesh) are logically lower than the complete fillet percentages usually found in the literature.

Chrisman et al. (1983) reported that dressout of tank-raised triploid channel catfish increased an average of 6.3%. These fish were three years old and were much older and heavier than fish in the present study. Pond-raised triploids in the present study, of typical commercial size and age, did not demonstrate similar processing advantages.

CONCLUSIONS

Economic benefit to the catfish industry would be significant if pond-grown triploid catfish had the same sterility-induced increase in dressout percentage that

Wolters et al. (1982) and Chrisman et al. (1983) found in 3-year-old tank-raised triploids. However, this did not occur in the present study. While significant differences were found in survival, yield, feed conversion and gonadosomatic indices between triploid and diploid channel catfish, none of these resulted in any economic benefit to catfish farmers. These differences would have resulted in a lower economic return from triploid catfish. Because of conflicting results from pond-raised versus tank-raised catfish, future research should focus on evaluating the performance from different strains, strain crosses, hybrid crosses and quantitative gene action in polyploid catfish.

SUMMARY

Summary of Methods

Channel catfish (I. punctatus), blue catfish, (I. furcatus), white catfish, (I. catus), and black bullhead catfish, (I. melas) were paired at the Auburn Fisheries Research Unit and the Louisiana Agricultural Experiment Station, in various combinations. The female parent was usually the channel catfish.

Cold shocks (5 C for 30 to 60 minutes at 10 min after fertilization) and pressure shocks (900-1500 kg/cm^2 for 5 minutes at 5 min after fertilization) were used to induce triploidy. Temperature-shock treatments to induce tetraploidy ranged from 38 to 42 C for durations of 3 to 6 minutes or 5 C for 45 minutes, while hydrostatic pressure treatments ranged from 4,000 to 8,000 psi for durations of 4 to 10 minutes. Shocks were initiated 43 to 90 minutes after fertilization. After the treatment, the embryos were immediately placed at the original incubation temperature. The first mitotic cleavage occurred approximately 80-90 minutes after fertilization. Treated eggs were incubated in aerated water at 26-27 C.

At 3 months age, fish were harvested, weighed to nearest gram, and analyzed for ploidy level. A sample of embryos or small fingerlings from each treatment was karyotyped to determine ploidy level. A total of 7 to 10 metaphase spreads was examined per slide and 1 to 5 slides were examined per replicate at 1000X.

Erythrocyte nucleus size was measured in 3-month-old fish by the sensing zone technique (Harvey 1968; Benfey et al. 1984) at AAES. A model ZM Coulter Counter (Coulter Electronics) and a model 256 Channelizer (Coulter Electronics) were used to determine the ploidy level in live specimens at AAES. A one-way analysis of variance was used to analyze percentage survival in heat shock treatments.

Randomly selected diploid and triploid hybrid fingerlings were stocked into six 1.3-m diameter fiberglass tanks at a rate of 8 per tank, for performance comparison. Three tanks were stocked with each treatment group. All bio-filtered tanks were filled with pond water. Water temperature and dissolved oxygen were measured daily using a Yellow Springs Instruments oxygen/temperature meter. Partial water exchanges (approximately 1/3 of tank volume) were performed weekly.

Growth, as measured by weight (to the nearest 1 g) and total length (mm), was recorded monthly for all fish. Dressout percentage ((fillet weight/total weight) x 100),

specific growth rate (g) (Krasnai and Marian 1986), condition factor (K) (Nielsen and Johnson 1983) and gonadosomatic index (Kaya 1972) were calculated for diploid hybrid and triploid hybrid catfish after a growout period of 150 days (18 months age).

Ovaries and testes from all diploid and triploid hybrid catfish were removed and fixed in Bouin's fluid at harvest. Tissue samples were dehydrated in an alcohol series and mounted in paraffin. Tissues were sectioned to a thickness of 6 μm and stained with hematoxylin and eosin. Slides were examined and photographed through a Zeiss photomicroscope.

In pond-growth experiments, fingerling diploid (63 ± 14 g) and and triploid (45 ± 17 g) channel catfish were stocked into 0.04 ha earthen ponds at a rate of 11,000 fish/ha. Three replicate ponds were stocked at each ploidy level. After a growout period of 185 days, all fish were harvested, counted, and weighed to nearest 1 g.

Fish were fed floating fish feed (32% protein) daily. The amount fed was calculated as a percentage of their body weight (DuPree 1984), and varied from 1 - 3% per day. Biomass estimates and feeding rates were updated monthly following the length/weight measurements. Feed efficiency was calculated from the grams of feed consumed divided by the grams of fish produced (Swingle 1959).

The significance level used in all statistical tests was $\alpha = 0.05$. Chi square was used to test for variation from the expected 1:1 sex ratio. Unpaired, two-way Student's t-tests were used to test for significant differences between most diploid vs. triploid characteristics. Two-way analyses of variance were used to test for interaction between ploidy and sex. Analyses of covariance were used to compensate for differences in stocking sizes of the hybrid treatment groups when testing for differences in growth between the ploidy levels.

Summary of Results

Temperature shock and hydrostatic pressure both induced polyploidy in ictalurid catfish. Hydrostatic pressure was more consistent than cold-shocks for inducing triploidy. Pressures of 1300-1500 kg/cm^2 for 5 min at 5 min after fertilization induced 100% triploidy with 50% hatching rate. Heat shock and hydrostatic pressure both induced tetraploidy. No tetraploidy was observed in fish subjected to heat shocks previously reported as effective for tetraploid induction in channel catfish. Treatments at 80-83 min after fertilization were the most effective for producing tetraploidy at 38-39 C. Tetraploids were subviable, as evidenced from decreasing survival from 3 days to 3 months of age. Diploid-

tetraploid mosaics with compressed bodies and caudal deformities were also produced.

In tank growout trials, covariance analysis of the weights of triploid hybrids and diploid hybrids indicated that the 2 groups had similar slopes *b* ($P = 0.954$) but different intercepts *a* ($P < 0.01$) due to differences in stocking weights. The specific growth rate "g" did not differ significantly between the 2 groups ($P = 0.133$) (Table I). Survival for both groups was 100%, with the exception of 1 tank of diploid hybrids (18 months age) lost to theft. Condition factor (K) for hybrid triploid catfish was significantly different ($P = 0.0321$) from that of the diploid hybrid catfish at 18 months of age. Feed conversion ratios (FCR) did not differ between the triploids and diploids ($P = 0.2342$). Mean feed conversion ratios for combined sexes of hybrid diploids and hybrid triploids were 2.6 ± 1.5 and 2.1 ± 1.2, respectively.

The gonadosomatic index (GSI) was not significantly different between diploid and triploid hybrid catfish ($P = 0.893$). Four out of eleven (36%) triploid hybrid females had a single ovary. Histology of diploid ovaries showed numerous developing oocytes. Histological examination of triploid hybrid ovaries revealed few normally developing oocytes and large numbers of abnormally dividing cells. Both testes were present in all male diploid and triploid hybrids. Triploid hybrid males had abnormal seminiferous tubules with no sperm present and were similar in appearance to triploid channel catfish testes. One triploid hybrid could not be sexed by gross examination of the gonadal tissue. Chi-square tests indicated the sex ratio did not differ significantly from the expected 1 : 1 in either diploid hybrids ($P = 0.705$) or triploid hybrids ($P = 0.756$).

Dressout percentage (analyzed using a 2-tailed t-test) did not differ significantly between the diploid and triploid hybrid treatments ($P = 0.069$), nor did it differ between the 2 sexes ($P = 0.393$). Dressout percentage varied more between the sexes of diploid hybrids (male $\bar{X} = 62.8 \pm 2.1$; female $\bar{X} = 68.7 \pm 13.9$) than between the sexes of triploid hybrids (male $\bar{X} = 62.2 \pm 4.8$; female $\bar{X} = 61.5 \pm 4.2$).

In pond growout trials, total weight of diploid and triploid channel catfish did not differ significantly at harvest ($p = 0.5959$). Mean weights for diploid and triploid catfish were 0.52 kg ± .03 and 0.54 kg ± .07, respectively. Yield and survival of triploid channel catfish were significantly lower ($P = 0.0245$ and $P = 0.0435$ respectively) than corresponding values for diploids.

Feed efficiencies were significantly higher for triploids channel catfish (P = 0.0436). Feed conversion ratios for pond-raised diploid and triploid channel catfish were 1.17 ± .15 and 1.37 ± .17, respectively.

The effect of reduced gonadal development was evident in the difference between diploid and triploid gonad weights and gonadosomatic indices (GSI). Gonads of triploid catfish were significantly smaller (P = 0.0352) than those of diploids, and this was especially evident in the gonadosomatic index (GSI) for female diploids and triploids (1.6 ± 3.0 and .71 ± .74 respectively). Triploid induced sterility had no significant effect on dressout percentages.

Summary of Conclusions

Results of tests of various methods point to the use of hydrostatic pressure as the method of choice for producing triploid catfish. Differential mortality of tetraploids compared to diploids could explain the low percentage of tetraploids observed as fish became older. Apparently, unknown factors, genetic or environmental, make certain ploidy level manipulations difficult to duplicate from one laboratory to another.

Triploid hybrids did not outperform diploid hybrid catfish in any of the growth, feeding, or dressout parameters measured. Significant differences in the condition factor (K) indicated that triploid hybrids were significantly heavier at any given length than the diploid hybrids, between 15 and 18 months age, inclusive. However, skeletal deformities were common among the triploids, and therefore the difference might be interpreted as the triploids being shorter, rather than of greater robustness.

No significant difference was found in the gonadosomatic index of diploid hybrids vs. triploid hybrids, even though over 1/3 of the triploid females had only a single visible ovary. It is suspected that the lack of significance is due to the immature age (19 months) of the study fish. Gonads were in an early stage of development in all fish at the time of examination. Histological examination revealed abnormal oocyte development, and severely reduced spermatozoa production in triploid hybrid catfish.

The value of triploid catfish to the industry remains to be proven. The desirable production characteristics which had been found in triploid channel catfish (Wolters et al. 1982) were not detected in channel catfish x blue catfish hybrids.

It could be of great economic importance if pond grown triploid catfish demonstrated the same sterility-induced increase in dressout percentage found by Chrisman et al. (1983) in 3-year-old tank-raised triploids. While significant differences were found between pond raised triploid and diploid channel catfish, none of these would be of any conceivable benefit to the catfish industry. At this time, first generation triploids and interspecific hybrids still remain difficult to produce in commercial quantities, and are unpredictable in their performance.

Further Research Needs

Growth and survival of triploid and tetraploid ictalurids needs to be further evaluated. Specifically, the effect of polyploidy-inducing treatments on different strains of catfish should be investigated. Tetraploids should be grown to maturity, their fertility and spawning behavior determined, the nature of their meiotic division studied,

and the performance of their progeny determined to establish the feasibility of using tetraploids for natural production of triploids or for combining the genomes of ictalurid catfishes through introgression.

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VITA

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Craig G. Lilyestrom

Major Field: Wildlife and Fisheries

Title of Dissertation: INDUCTION OF POLYPLOIDY IN ICTALURID CATFISH AND COMPARATIVE PERFORMANCE OF DIPLOID AND TRIPLOID CHANNEL CATFISH AND CHANNEL X BLUE CATFISH HYBRIDS

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