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INDUCED TRIPLOIDY IN GRASS CARP, *CTENOPHARYNGODON IDELLA* VAL.

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ABSTRACT

Cassani, J.R. and Caton, W.E., 1985. Induced triploidy in grass carp, *Ctenopharyngodon idella* Val. *Aquaculture*, 46: 37—44.

Induction of triploidy in grass carp was accomplished by means of thermal shocks to eggs shortly after fertilization. Triploidy occurred most often with cold shocks at 5—7°C and at durations of 25—30 min starting 2.0—4.5 min after fertilization. Estimated percent triploid ranged from 50 to 100% on five occasions. With one exception, cold shocks of 5—7°C for less than 25 min did not induce triploidy, and cold shock durations of 30 min or longer generally resulted in 100% mortality. A heat shock of 40°C for 1 min, 4.75 min after activation, was the only heat treatment which produced triploidy (8%) with 81% surviving to the blastula stage. Fertilized eggs immersed in a solution of cytochalasin B (10 mg/l, 0.1% DMSO) for 10 min, 12 min after activation, resulted in 54% of the eggs surviving to the blastula stage with none found to be triploid.

INTRODUCTION

Induction of triploidy has been successfully accomplished in several species of fish through a variety of techniques. The primary objective of such research is to produce fish that are theoretically incapable of reproduction and, as a possible indirect effect, have a faster growth rate, better feed efficiency and in some species less mortality at sexual maturity than normal diploids. Inability of homologous chromosomes to synapse properly during gametogenesis is thought to be the cause of functional sterility in triploid individuals (Stanley, 1979).

Thermal shocks involving immersion of fertilized eggs in a hot or cold water bath shortly after fertilization have been used to induce triploidy in several species (Swarup, 1956; Valenti, 1975; Gervai et al., 1980; Hoornbeek and Burke, 1981; Wolters et al., 1981). However, the timing temperature and duration of the thermal shock must be determined for each species (Thorgaard, 1983). Salmonid eggs subjected to various concentrations of cytochalasin B and colchicine have also resulted in polyploid offspring (Refstie et al., 1977; Allen and Stanley, 1979; Refstie, 1981).

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The grass carp (*Ctenopharyngodon idella* Val.), a widely introduced species, is of value as a food fish and as a biological control agent for aquatic weeds (Bardach et al., 1972; Sutton, 1977). However, grass carp has been banned from many areas due to its potential for reproduction. The objective of this study was to determine if triploidy could be induced in grass carp using traditional thermal shock techniques and if so, what combinations of temperature, exposure time, and time of initiation would produce the best results.

MATERIALS AND METHODS

Grass carp spawning operations begin in southwest Florida during April when water temperatures reach 24—25°C. Hypophysation of mature female broodfish (age VII and VIII) was accomplished with LHRH (luteinizing hormone releasing hormone Des-Gly^{10}-[D-Ala^{6}] LHRH ethylamide) obtained from Sigma Chemical Co. A standard dose of 10 μg/kg was given intramuscularly 14—18 h before the expected stripping time. After 7 May when pond temperatures reached 28—30°C, female broodfish were transferred from holding ponds to a 6000-l indoor tank and maintained at 23°C ± 1°C 10—20 days prior to the first hormone injection. Hypophysation of female grass carp maintained in this manner was accomplished by two injections of human chorionic gonadotropin (HCG) (440 IU/kg first injection and 1870 IU/kg second injection) 24 h apart and a third injection of carp pituitary (9.9 mg/kg) 24 h after the second HCG injection. Two females were stripped in April using LHRH and the remaining seven females were stripped using the HCG/carp pituitary method during May and June.

Stripping and fertilization was done according to the dry method (Rothbard, 1981). Approximately 1—2 min after egg—sperm activation, eggs were placed in cylindrical saran baskets with PVC frames and were immersed in cold (5—10°C) and hot (39—40°C) baths for various durations and initiation times after fertilization. Cold water was maintained with a thermostatically controlled water chiller—circulator, and in situations where eggs were acclimated to the cold shock (temperatures preceded by ∿, i.e. ∿7°C) ice was used to gradually lower the temperature to the target temperature as close to 5 min after egg—sperm activation as possible. Hot water baths were maintained with a thermostatically controlled water heater—circulator. No attempt was made to quantify the eggs per treatment due to problems in handling from the lack of adequate time between egg—sperm activation and the beginning of the thermal shock. On one occasion, eggs were immersed in a solution of cytochalasin B (10 mg/l and 0.1% DMSO) for 10 min, 12 min after egg—sperm activation. Treatment eggs and controls were incubated in cone and/or funnel shaped containers at less than 1000 est. eggs/l, receiving a minimum of 2 l/min fresh aerated, filtered and UV light sterilized well water.

The percentage of viable eggs (compared with the controls) remaining

6—8 h after egg—sperm activation (early to mid-blastula stage) was determined by examining a minimum of 50 eggs at 15× magnification. Ploidy was determined by counting chromosomes per spread for 12 larvae from each treatment. In situations where less than 1% survival was estimated, then only six chromosome preparations were prepared per treatment.

Chromosome spreads were prepared by immersing larvae in a 0.01—0.03% solution of colchicine for 16—20 h. Individual larvae were then mascerated in a hypotonic solution (1.0% sodium citrate and distilled water) with a scalpel and 45 min later centrifuged for 5 min at 750*g*. After centrifugation and decanting the hypotonic, cellular pellets were fixed by adding 3 : 1 methanol—acetic acid and refrigerated for 30 min. Final cellular suspensions were dropped onto cold slides, air-dried and stained in 10% Giemsa. Four to 10 chromosome spreads that were isolated and exhibited good resolution were counted per preparation.

RESULTS

Of the nine grass carp artificially spawned during the 1984 season, six (67%) produced eggs resulting in triploid offspring after thermal inducements. Triploidy was induced at 5—7°C for durations of 6 and 25—31.5 min starting 2.0—4.75 min after egg—sperm activation. Cold shocks of 30 min or longer generally resulted in 100% mortality. Considerable variation

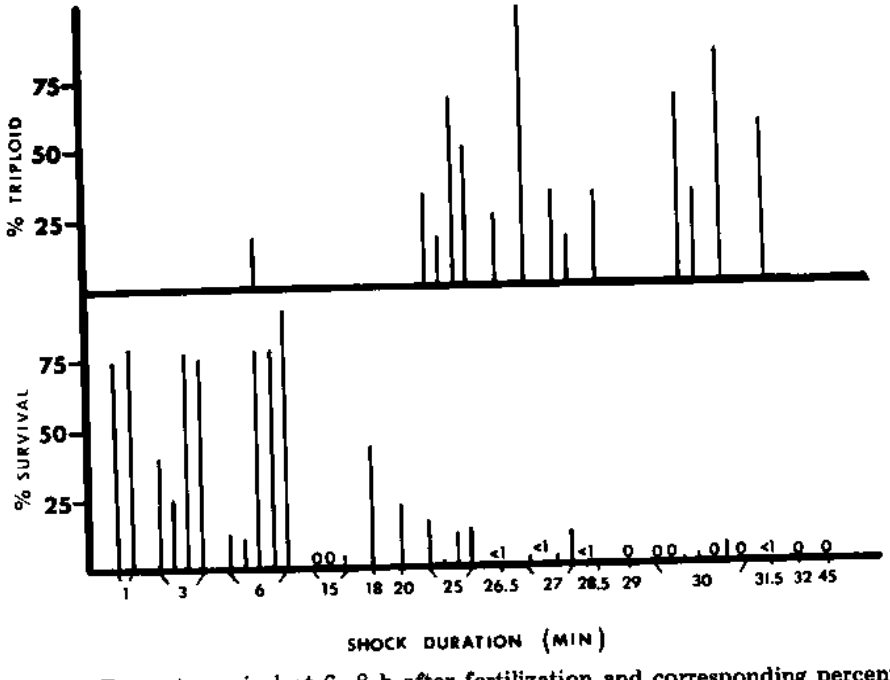

Fig. 1. Percent survival at 6—8 h after fertilization and corresponding percent triploidy for cold shocks of 5—7°C.

TABLE I

Thermal shock treatments and estimated survival and triploid induction

Treatment (°C)	Ambient temp. (°C)	Time after activation (min)	Duration (min)	% Viable[a]	% Triploid	Date[d]	% Viable[a] control
4	23.5	1:00	3:00	40	0	04 April	42
4	23.5	1:00	6:00	12	0	04 April	42
4	23.5	1:00	15:00	0	—	04 April	42
4	23.5	1:00	30:00	0	—	04 April	42
4	23.5	4:00	3:00	26	0	04 April	42
4	23.5	4:00	6:00	11	0	04 April	42
4	23.5	4:00	15:00	0	—	04 April	42
4	23.5	4:00	30:00	0	—	04 April	42
5	23.8	4:45	1:00	75	0	15 April	82
5	23.8	4:45	3:00	78	0	15 April	82
5	23.8	4:45	6:00	78	18	15 April	82
5	23.8	13:00	1:00	80	0	15 April	82
5	23.8	13:00	3:00	76	0	15 April	82
5	23.8	13:00	6:00	79	0	15 April	82
40	23.8	4:45	1:00	81	8	15 April	82
40	23.8	14:00	1:00	73	0	15 April	82
39	23.0	4:00	1:00	80	0	17 May	>80
39	23.0	3:00	1:00	74	0	17 May	>80
5	23.0	4:30	6:00	92	0	24 May	99
5	23.0	4:30	15:00	9	0	24 May	99
5	23.0	4:30	30:00	2	67	24 May	99
5	23.0	4:30	45:00	0	—	24 May	99
39	23.0	4:00	1:00	95	0	24 May	99
39	23.0	4:30	1:00	99	0	24 May	99
39	23.0	5:00	1:00	93	0	24 May	99
5	23.0	4:00	20:00	21	0	02 June	87
5	23.0	4:00	25:00	16	33	02 June	87
5	23.0	4:00	27:00	3	100[b]	02 June	87
5	23.0	4:00	30:00	1	33	02 June	87
9—10	23.0	4:00	25:00	10	0	02 June	87
9—10	23.0	4:00	30:00	4	0	02 June	87
9—10	23.0	4:00	35:00	11	0	02 June	87
9—10	23.0	4:00	40:00	12	0	02 June	87
5	23.5	4:00	25:00	2	17	11 June	—
5	23.5	4:00	27:00	<1	0	11 June	—
5	23.5	4:00	30:00	0	—	11 June	—
∿5[c]	23.5	2:30	26:30	<1	25	11 June	—
∿5	23.5	2:30	28:30	<1	33	11 June	—
∿5	23.5	2:30	31:30	<1	58	11 June	—
∿5	23.0	2:00	29:00	0	—	15 June	>80
∿5	23.0	2:00	32:00	0	—	15 June	>80
∿7	23.0	2:00	18:00	41	0	20 June	53
∿7	23.0	2:00	25:00	12	67	20 June	53
∿7	23.0	2:00	27:00	4	33	20 June	53
∿7	23.0	2:00	30:00	7	83	20 June	53
∿7	23.0	2:00	25:00	13	50	25 June	94
∿7	23.0	2:00	27:00	12	17	25 June	94
∿7	23.0	2:00	30:00	0	—	25 June	94
∿7	23.0	13:00	25:00	27	0	25 June	94
∿7	23.0	13:00	30:00	7	0	25 June	94
39	23.0	4:30	1:00	65	0	25 June	94
39	23.0	4:30	2:00	3	0	25 June	94
39	23.0	4:30	5:00	1	0	25 June	94

[a]Survival at 6—8 h after egg—sperm activation (blastula stage).
[b]Eggs from second stripping, 51 min after first stripping.
[c]Acclimated to the target temperature ±1°C before 5 min after egg—sperm activation.
[d]Different dates indicate results from different females.

in survival and percent triploidy occurred between eggs from different females. However, a sharp decline in survival was evident at cold shock durations of 25 min or longer (Table I; Fig. 1). Induction of triploidy at cold temperatures appeared to be directly related to cold shock duration and inversely related to survival (Fig. 1). The best cold shock combinations based on percent triploidy and survival were 5°C, starting 4 min after egg—sperm activation, for 25, 27 and 30 min, and at 7°C acclimated, starting 2 min after egg—sperm activation, for 25, 27 and 30 min (Table I).

Heat shocks of 39--40°C starting 3—5 min after egg—sperm activation produced triploid offspring (8%) on only one occasion (Table I). Heat shocks of 39°C for 2 min on 25 June resulted in only 3% of the eggs surviving to 8 h, all of which were estimated to be diploid. Eggs from the same batch shocked at 39°C for 5 min resulted in 100% mortality. Most cold and heat treatments, especially at the longer durations, resulted in 5—10% of the larvae being deformed, which makes the figures for percent viable at the blastula stage an overestimate.

The use of cytochalasin B on 24 May resulted in 54% of the eggs surviving to 8 h. No triploid larvae were found among the random sample of 12 from this treatment.

DISCUSSION

The most common mechanism involved in the production of triploid fish is suppression of the second meiotic division in the fertilized egg or suppression of an early mitotic division in the fertilized egg (Thorgaard, 1983). Triploidy in grass carp ♀ × bighead ♂ hybrids was determined to be the result of second polar body retention based on karyotypic techniques (Beck et al., 1980; Cassani et al., 1984). The timing of the shock treatment must coincide near or at the time of the second meiotic division which in grass carp is thought to be near or at 5 min after fertilization (Bobrova, 1969). The effect of the ambient temperature and its relation to the time of second meiotic division initiation after fertilization is not clearly understood. Stanley (1979) reported the best results for heat shock induction of diploid gynogenesis in grass carp at 2—5 min after fertilization (4.7%). The highest percentage of viable triploid carp (*Cyprinus carpio* L.) fry was attained for those eggs cold shocked 5 min after fertilization (Gervai et al., 1980). Cold shock induction of triploidy also occurred at 5 min after fertilization in channel catfish (Wolters et al., 1981).

The cold shock temperatures were arbitrarily started for the first fish at 4°C and were gradually increased in an effort to increase survival and the percentage of triploid offspring. Shocking eggs at 9—10°C did not noticeably increase survival or triploidy at 25 min duration (Table I). Acclimating eggs to the targeted cold shock temperature had no discernible effect on survival or triploid induction compared with similar treatments with abrupt temperature shocks because of different responses between females (Table I).

Egg ripeness may be a factor in poor survival at the longer cold shock durations. Five of the nine females were spawned during the month of June, which is considered the last month for artificial spawning of grass carp at this location (26° North latitude). However, maintaining these females at 23°C for 10—20 days prior to spawning, a 6° or 7° drop from pond temperatures, may have temporarily halted readsorption of the eggs and overripeness. Differences in egg ripeness may be responsible for contrasting results involving second polar body retention (Lincoln et al., 1974; Refstie et al., 1982), but the controls from the late season fish had a high level of survival and hatchability, which are not indications of overripeness (Rothbard, 1981; Zonneveld, 1984). One of the two fish spawned during the month of April, considered to be the best month for spawning in southwest Florida, produced eggs resulting in 18% triploidy and 78% survival with a cold shock of relatively short duration (6 min at 5°C). The only heat shock resulting in triploid offspring was also from this same batch of eggs, probably indicating optimal ripeness.

A genetic tendency toward polar body retention or susceptibility to thermal shock may be a factor for inconsistent results between these and other data. Distinguishing females which consistently produce eggs susceptible to thermal shock can be important for improving survival and increased production of triploid progeny. However, several seasons of testing this hypothesis would be necessary.

Stripping time is another factor affecting polar body extrusion. Zonneveld (1984) reported that if stripping is delayed grass carp eggs become overripe, resulting in poor fertilization and hatching. According to Horvath (1978), there is an optimal period after the last hormone injection of 1/2 to 3/4 h in the temperature range 20—26°C in which the eggs should be stripped. Stripping time may have been related to the treatment resulting in an estimated 100% triploid induction (Table I). On this occasion the female was stripped a second time 51 min after the first stripping, suggesting that eggs from the first stripping may not have been "optimally ripe."

Our results for triploid induction are not suitable for grass carp for food production due to the relatively low survival and inter-female/egg variations in percent triploid (Table I). However, these methods can be used for producing a limited number of sterile grass carp for aquatic vegetation control where grass carp reproduction is of concern. Reasonably rapid segregation (200 fish/h) can be achieved with the use of a Coulter Counter® and a small volume of erythrocytes from each individual (Wattendorf, in press). In treatments resulting in 30% or greater triploid offspring, a large number of sterile fish can be acquired in a short time. Because the grass carp is a prolific fish, with typically one million eggs or more in an 11—12 kg female, a large batch of eggs resulting in 5% survival and 30% triploidy could yield approximately 15 000 triploid fry. Allowing for as much as 30% mortality from deformed embryos and predation, over 10 000 fish would be produced.

Additional research, especially with regard to egg ripeness and its relation-

ship to thermal shock susceptibility, and further work with heat shocks will be required for better survival and consistency of results.

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