MONOSEX TILAPIA PRODUCTION THROUGH ANDROGENESIS

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ABSTRACT

Control of reproduction is vital to aquaculture and includes artificial propagation as well as management of unwanted recruitment. Developments in manipulation of the reproductive system provide options to enhance production. Nile tilapia, *Oreochromis niloticus*, spawning was managed by photoperiod and temperature manipulation. A controlled light cycle of 20L:4D and water temperature of 26 ± 2°C directed spawning to a predictable time frame. A developmental rate (t_0) relationship was described and applied to chromosome manipulation. Blond Nile tilapia are homozygous recessive for a color mutation that was used as a phenotypic marker in the development of protocol for androgenetic induction, while the color pigmentation for red Nile tilapia is dominant over the wild type color pattern. Androgenotes were produced by neutralizing the female genome of normal color Nile tilapia or that of red tilapia (600 J m^{-2} UV dose), eggs were activated with sperm from blond males or Ghana males, respectively, and then the eggs were diploidized with cold shock (11 ± 0.5°C for 60 min) applied at various times after incubation at 28 ± 0.2°C. Shock applied at 69 min post-activation produced greater numbers of androgenotes than shocks applied at 59 or 79 min post-activation; the shock application time of 69 min was used for induction with red tilapia stocks. Production of viable diploid androgenotes for crosses involving either red or blond and Ghana stocks was very low, and no progeny survived to maturity. Thus, neither verification of sex determination in androgenotes nor testing of monosex breeding was accomplished.

INTRODUCTION

Management of fish reproduction is important to aquaculture, as one of the major constraints to aquaculture development is the reliability and sustainability of seed organisms for culture. Reproductive management may involve production of seedstock through artificial propagation or, conversely, through development of limits to recruitment. Enhancement of spawning can be by habitat manipulation or through direct hormonal intervention, while reproductive control also can be direct or based on more complicated modifications (Shelton, 1989; Dunham, 1990). Within the context of tilapia farming, this limitation involves effective and practical control of unmanaged recruitment. Tilapia culture is one of the fastest growing forms of finfish aquaculture in the world. Production efficiency and product marketability in most economies depend on the capacity to control unwanted reproduction. Various approaches to monosexing fishes have been applied to culture. Techniques for managing recruitment have evolved from traditional means (predator control, hybridization, or hand-sexing) to the current practice of monosexing by steroid-induced sex reversal, which has been the industry standard during the past couple of decades. However, chemical treatment of food fish has become increasingly constrained, and since this tool could be withdrawn by governmental regulation, an alternative should be available. The use of chromosome manipulation is one of the more recently implemented practices (Shelton, 1989; Dunham, 1990).

Chromosome manipulation, which includes ploidy alteration or euploidy induction with single-parent genome contribution, can provide alternative control mechanisms. Ploidy manipulation includes the induction of triploids through polar body retention or tetraploidy through interference with first zygotic mitosis. Euploid alterations include gynogenetic (gynogenote) diploidization of the maternal genome through polar body retention (meiogynote) or mitotic interference (mitogynote). Gynogenesis has been investigated widely, but the development of techniques to induce androgenesis, i.e., progeny with only a paternal genome, is much less studied (Thorgaard and Allen, 1986). Diploidization of the paternal genome (androgenote) is accomplished by interference with first mitosis for eggs that have been genome-neutralized before activation. Androgenesis can provide a mechanism for the development of unique broodstock to use in producing all-male progeny without the use of sex-reversing steroids.

Within the contemporary atmosphere of increasing governmental regulation on the use of chemicals on food fish, continued dependency on steroid-induced monosexing places the culture of tilapias in a precarious position. The recently developed protocol to develop YY-male tilapia relies on estrogen treatment, and even though fish to be cultured are one generation removed from the treatment, the protocol still depends on progeny testing to identify target broodstock (Scott et al., 1989; Mair et al., 1997). In contrast, androgenesis offers the potential of direct induction for YY-male tilapia, without chemical treatment and ultimately without the need for progeny testing to identify the unique male broodstock. Although the genetics of sex determination are based on an incompletely characterized system (Wohlfarth and Wedekind, 1991; Mair, 1993), the perceived complications might be minimized by using select strains ("Monosex Tilapia Production through Androgenesis: Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production," 9RCR6A; pp. 39–43 of this volume). A concurrent collaborative PD/A CRSP–funded study at Auburn University, Alabama, (R. Phelps, principal investigator) is examining strain varia-

tions in sex ratio inheritance. Androgenotes from the present study were to be progeny-tested (males and females) along with other strains as a component of examining the genetic basis of sex determination.

Assuming that sex determination is effected by control from a single pair of chromosomes, a monosex breeding program using YY-male broodstock might provide such a solution. Through androgenesis, YY-males can be induced directly, independent of steroids, and after verification, used without the need for subsequent progeny-test identification. Sex determination is characterized by a homogametic/hetero-gametic genotype; however, autosomal modifier genes may alter the theoretical 1:1 progeny sex ratio (Shelton et al., 1983; Wohlfarth and Wedekind, 1991; Mair, 1993). Therefore, deviations in the expected all-male progeny from YY-male breeding should be anticipated. Thus, this study proposed to investigate a protocol to produce androgenotes (progeny with only a paternal genome) and also to examine the basic mechanism of sex determination.

Androgenesis should result in offspring of equal sex ratio; females would be XX and males would be YY. Progeny testing will be required during experimental development to confirm that the males are fertile and that only male offspring (XY) will result when spawned with normal females (XX). Sex ratio of progeny from crosses of androgenote females with normal males should be 1:1, and presumptive YY-male androgenote crossed with normal females would be expected to produce only male progeny; these results would verify the assumptions of monochromosomal sex determination. The YY-males would then be a basis for developing a unique broodstock that would produce all-male progeny and add insight into the stability and fidelity of the sex-determining system in tilapia.

Tilapias do not respond well to hormonal induction for spawning control, but interruption of aquarium spawning so as to collect gametes might be sufficient to proceed with chromosome manipulation studies. Chromosome manipulation involves one or two basic treatments after obtaining fresh gametes (Thorgaard and Allen, 1986). For androgenesis the first treatment is the deactivation of the female genome. Ultraviolet (UV) irradiation is preferred for simplicity and safety but also because it dimerizes the DNA rather than fragmenting it. Egg activation with untreated spermatozoa then requires diploidization of the haploid zygote by some form of shock to interrupt the first mitotic division. Shock is most often physical, e.g., thermal (cold or hot) or pressure. Thermal treatment is usually preferred because of the ease of application and equipment simplicity. In order to prevent chromosome segregation, the shock must be timed to coincide with a cytological event, such as disruption of the spindle fibers during metaphase to prevent karyokinesis, or inter-ference with the cell duplication during cytokinesis. Thus, shock type and intensity, duration, and time of application must be optimally combined into a protocol for maximum yield of diploid progeny. Further, because the rate of develop-ment is inversely temperature dependent, either the preshock incubation temperature must be standardized or the shock time must be calibrated to account for the temperature effect. Absolute shock time (minutes post-activation) can be trans-formed with reference to an index of development rate or mitotic interval (τ_0), also in minutes (Dettlaff and Dettlaff, 1961). Shock time (τ_s) can be related to tau (τ_s/τ_0) to report shock protocol in a dimensionless index, which is temperature

compensated (Dettlaff, 1986). A tau curve for the Nile tilapia and gametic treatment with UV were described during an earlier segment of this study (Shelton, 1999), and one phase of the diploidization protocol was reported (Shelton, 2000). Both gametic treatments (UV and shock) are near lethal, increasing direct mortality, and further, the genomic diploidization will increase homozygosity, thereby reducing fitness. Thus, survival of viable androgenotes to maturity is expected to be quite low. This report describes the initial development of a protocol for the production of androgenetic progeny of Nile tilapia, *Oreochromis niloticus*.

METHODS AND MATERIALS

Broodstock

The University of Oklahoma stock of *O. niloticus* used in the earlier study segments was obtained from Auburn University, Alabama, in 1982, and it originated in Ivory Coast. The blond stock was derived from a population in Lake Manzala, Egypt (Scott et al., 1987; McAndrew et al., 1988) and was obtained from the University of Wales, Swansea, in 1987. Stock of the red mutant was also originally derived from Lake Manzala, Egypt, and a founder population for our work was obtained from Israel as about 50 juveniles in the fall of 1997. Broodstock development was delayed until 2000. The red color mutation is dominant to the wild pigmentation (McAndrew et al., 1988). During various studies the Ivory Coast population became hybridized to an unknown level with the blond stock. Since this is the phenotypic marker that was to be used in our study, a different stock was obtained for the final series of experi-ments. Nile tilapia from a Ghana source were obtained from Auburn University in 1998, but the fish in this founder stock were small. They were transferred to ponds for the summer to mature, then overwintered. Fish from this population were first used in experiments beginning in January 1999. Each stock (Ghana, blond, and Ghana × blond) was progeny-tested for color phenotype at developmental stages from hatching to swim-up; progeny testing of red stocks for color inheritance began in 2000.

Two basic developmental questions were initially addressed. One of these was the pigment pattern ontogeny in the various stocks—Ivory Coast, Ghana (G), and Egyptian (E)—and the two color mutations, blond (Bl) and red (R), used in the experiments that were developed from Egyptian stock (McAndrew et al., 1988). In the trials to induce androgenesis, one of the two gamete donors (male or female) was from one of the two color mutants. Pigment development was examined in the various stocks (IC, G, Bl, R) and their hybrids. The sequence of pigmentation provided a means of differentiating between normally fertilized offspring of IC or G or their hybrids with color mutants and larvae with only the genome of the color mutant stock (phenotypic marker). This latter would identify androgenote progeny as distinct from any offspring resulting from normal fertilization.

Spawning

Spawning was managed through regulation of photoperiod (20L:4D) and temperature (26 and 36°C), but this provided only limited control (Shelton, 1998, 1999). Although injection with gonadotropic materials is reported to lead to predictable ovulation (Gissis et al., 1988), we were not able to regularly duplicate this success. Therefore, behavior was observed so as

to identify receptive females, and then stripping was accomplished in those instances when active courtship and spawning resulted; spawning was interrupted, and the eggs were collected from these females. Several females (four to six) were stocked with one male in four large aquaria (550 liters) or paired in 200-liter aquaria. Water was aerated and was circulated at the rate of one turnover per day, and temperature was maintained at $26 \pm 2°C$. The light cycle was controlled by adjusting a timer to turn on the overhead lighting at 0100 hours; the natural sunset regulated the end of the photoperiod for a total of about 20L:4D. Tilapia spawn about 8 to 10 h after the beginning of the light cycle (Myers and Hershberger, 1991). Ovulation and spawning readiness were judged by courtship behavior, coloration, and papilla erection (Rothbard and Pruginin, 1975; Rothbard, 1979). Females were stripped after initiation of spawning or as indicated by other characteristics that signal ovulation. Artificial propagation of tilapia includes gamete stripping, *in vitro* fertilization, and surrogate incubation; each component results in lower survival compared to normal spawning and *in vivo* incubation by the female (Rana, 1988).

Fertilization

For developmental rate experiments, eggs were collected in a clean container, milt was expressed over the eggs, and water was added directly to initiate activation (Rana, 1988). Fertilized eggs were placed in incubation chambers submerged in a water bath within 2 to 3 min, temperature was regulated closely, and 20 to 30 eggs were examined under magnification at intervals of 5 min to record cleavage rate. Developmental rate (τ_0) is defined by the duration of one mitotic cycle during early synchronous cleavage (Dettlaff and Dettlaff, 1961). Tau curves have been used to facilitate chromosome manipulation studies in various fishes (Shelton and Rothbard, 1993; Shelton et al., 1997). The tau curve of Nile tilapia was reported in an earlier progress report (Shelton, 1999); it was described from mean time intervals between the initiation of the first and third mitoses in 5 to 10% of the eggs over a range of temperatures (20 to 30°C). Subsequently, eggs were incubated at $28 \pm 0.2°C$ in upwelling units of one liter capacity with sufficient flow to gently tumble the developing embryos. For progeny testing of pigment development, fertilized eggs from natural spawns as well as from *in vitro* fertilization were used; observations on the appearance and pattern of melanophores were recorded at prehatching, posthatching, and at swim-up stanzas for Ghana, blond, Ghana × blond, red, and both reciprocal crosses with Ghana. Fish occasionally spawned outside of periods of observation, but fertilized eggs were collected from the brooding females, moved to incubators, and pigmentation development observed.

Chromosome Treatment

Freshly ovulated eggs were stripped from normally pigmented Ghana females and allocated into four subsamples. Two to four hundred eggs (about 2 ml) were placed in each of four 10-cm petri dishes with enough 28°C water (10 ml) to provide slight buoyancy. Three subsamples were placed in a UV-crosslinker (FisherBiotech, FB-UVXL-1000) and exposed to 600 J m^{-2} UV; this dosage was determined in an earlier study to be sufficient to dimerize the female genome (Shelton, 1999). The UV-treated eggs were activated with freshly collected spermatozoa from a blond male, then maintained at 28°C on a rotating shaker table; the untreated eggs (control) were fertilized with milt from the same male. After 30 min all eggs were transferred from the petri dishes to screened, individual incubator chambers, which were submerged in a 28°C water bath. Treated eggs were subsequently plunged into water in a thermal bath at $11 \pm 0.5°C$ for 60 min; one subsample was cold shocked at 59 min post-activation, the second at 69 min, and the third at 79 min. The first shock time coincided with the initial mitotic metaphase, the second just prior to cleavage furrow formation (cytokinesis), and the third immediately after cell division. After shock all eggs were returned to the water bath, and then they were transferred, including controls, into individual flow-through incubators. Development was monitored through hatching and swim-up. Beginning in 2000, red female broodstock were used as the egg donor and were fertilized (control) or activated (androgenesis) with sperm from Ghana males.

RESULTS AND DISCUSSION

Developmental Rate

Various experiments were conducted to measure developmental rate; 23 observations between 20 and 28°C established a tau curve relationship that permitted adjusting shock time to compensate for incubation temperature differences. For all experimental treatments, a replication as a control was included. Twenty-three tests of genome deactivation by UV-egg treatment at 400 to 600 J m^{-2} resulted in complete DNA dimerization. Spermatozoa from males of one of the color mutants was used to activate treated eggs. Twenty-three trials were used to optimize diploidization of the haploid male genome with a shock of 28°C for 60 min at 59 ($1.8\tau_0$), 69 ($2.1\tau_0$), and 79 ($2.4\tau_0$) min post-activation at 28°C. The time of shock initiation was adjusted for incubation temperature using a developmental rate curve, or tau (τ_0) curve (Shelton, 1999). Gamete treatment with UV has been the mechanism used to remove the maternal genomic influence. Egg treatment for androgenesis is more difficult than treating spermatozoa in gynogenesis because of the difference in cell size and because of the complication of orientation with reference to nuclear position during exposure. A dose rate of about 600 J m^{-2} was determined to be effective to dimerize the egg DNA. After shock, eggs were hatched in flow-through incubators. The hatch rate of androgenotes was very low, and none of the androgenotes survived beyond juvenile stages. Thus, none reached maturity so that the expected sex ratio of 1:1 could be verified, nor could progeny testing of presumptive YY-males be accomplished so as to determine whether only male progeny would be produced.

Spawning and Progeny Testing

Photoperiod manipulation permitted spawning activity to be forced to mid-day. During experiments prior to ploidy studies, a total of 86 natural spawns and 87 strip spawns resulted in 44 and 16% hatch rate, respectively. Females ovulated and spawned between about 9 and 14 h (mean = 11.8 h) after the light-on cycle. Ovulation based on the light cycle was a reasonable means of predicting stripping time. Hormonal induction of ovulation would seem to be a logical extension, but cichlids have responded poorly to gonadotropic therapy (Rana, 1988). During the summer of 1998, while the Ghana broodstock were in ponds, progeny testing of blond stocks continued. Beginning in January 1999, the Ghana stock was incorporated in the spawning efforts. No spawning occurred among the Ghana stock until April, then progeny testing was initiated.

Five spawns were sufficient to establish the developmental pattern of pigment for this race. The appearance of melanophores in larval tilapia from normally pigmented broodstock is clearly differentiated from that for blond progeny; 26 crosses were made with red and Ghana broodstock (R female × G male, G female × R male , R female × R male), but only 16 resulted in progeny (Table 1). Again, the sequence of pigment development permitted identification of androgenotes from as early as prehatch embryos to swim-up; juvenile and adult pigment of red tilapia is entirely distinct from that of normal tilapia. The sequence of melanophore development in three areas is useful for identifying the parental source of progeny; these areas are the yolk, eye, and brain surface. Progeny of normally pigmented stocks (Ghana) developed melanophores first in the eyes and on the yolk surface; pigment cells could be seen 1 to 2 d prior to hatching. Eye color appeared about 12 h prior to hatch but was initially diffuse and then developed as a gradual blending of melanin and gold pigmentation. At hatching scattered chromatin-filled melanophores were present on the dorsal surface of the brain and laterally on the body, adjacent to the notochord and near the yolk sac, but subsequently, the melanophores progressed caudad and gradually increased in abundance. In contrast, progeny of blond stocks totally lacked pigmentation in these areas until post-hatching, and then coloration was limited to a gold cast in the eyes. Body color did not develop during the larval stages, and only non-pigment bearing melanophores could be seen on the yolk and brain surfaces. These appeared as shadowy cellular outlines that contained no melanin. Finally, hybrids between pigmented females (Ghana) and blond males developed pigmentation in the areas and in the sequence described for the Ghana stock. Thus, progeny that have dark coloration in the first several days post-hatching (until the mouth becomes functional) and

possess genomic contribution from one or both pigmented parents, but not exclusively from the blond parent(s). The latter are non-pigmented throughout larval states. Pigment of the red color mutant is dominant in crosses with normally pigmented fish; development is first in the eyes, but pale and diffuse melanophores appear on the dorsal brain surface and lateral yolk. No permanent melanistic pigment develops subsequently; juveniles and adults are predominantly light colored. Androgenotes derived from blond male parents or red females can therefore be easily identified from pre-hatching through swim-up stages.

Chromosome Manipulation

Fertilization rate has been variable; in the six crosses involving red tilapia for androgenetic induction, some eggs from all crosses hatched (Table 2), but in the crosses with blond broodstock, 6 of 17 batches that were strip-spawned (artificially fertilized) from Ghana females had no development in controls, but in the 11 other strip-spawned groups that were artificially fertilized, the hatch rate in controls ranged from only 4.0 to 11.3% (mean 10.9%). Motility of spermatozoa was evaluated microscopically and was used only if more than 90% of cells were viable. Poor quality of ova is most often the primary cause of developmental failures. Initiation of egg resorption is common among tilapias, and although it does not prevent ovulation of the bad eggs, fertilization and hatching are poor (Peters, 1983). However, some physiological characteristics of tilapia gametes provide advantages in chromosome manipulation. Eggs retain high fertility for 3 to 6 h post-immersion in water (Myers et al., 1995), and sperm remain motile in water for several hours, in contrast to most fishes (Yeheskel and Avtalion, 1986).

Table 1. Pigment development during ontogeny of *Oreochromis niloticus* (G – Normal, Ghana; Bl – Blond, Egyptian; R – Red, Egyptian; functional lower jaw at swim-up) (+++ heavy melanophore pigment; ++ moderate; + light pigment; 0 no pigment; g gold pigment; melanophores gradually proliferate from initial scattered cells). Female is listed first in each of the progeny test crosses.

Stage at 28–30°C	Anatomical Feature	G × G	G × Bl	Bl × Bl	R × R	R × G	G × R
Pre-Hatch	Eye	++, g	0	0	+, g	+, g	+, g
	Head	0	0	0	0	0	0
	Yolk	++	++	0	0	0	0
Hatch	Eye	+++	+	0	++	++	++
	Head	++	+	*	+	+	+
	Yolk	+++	++	*	+	+	+
Swim-Up	Eye	+++	++	*	++	++	++
	Head	+++	++	*	+	+	+

* Empty cells but with no melanistic pigment.

Table 2. Summary* of induction trials to produce androgenetic Nile tilapia using UV-treated eggs (600 J m^{-2}) from Ghana strain females inseminated with sperm from blond (Egyptian strain) tilapia and diploidized by shock (11 ± 0.5°C for 60 min) at 1.8, 2.1, and 2.4τ_0.

Total Eggs	Control Hatch (N, %)	1.8τ_0 Hatch (N, %)	2.1τ_0 Hatch (N, %)	2.4τ_0 Hatch (N, %)	Survival to Maturity (N)
6,655	257, 16%	16, 6%**	103, 40%**	38, 14%**	0

* Data from 22 trials as reported in Shelton (2000).
** Note – Calculation error in published report (Shelton, 2000) for percent hatch, which is relative to the control; replicates were equal numbers of eggs subdivided into four groups (control and three treatments), and the original calculations used total egg number per batch as divisor rather than number hatched in the control.

Hatch rate of UV-treated and cold-shocked ova was significantly lower than that of controls, i.e., never more than a fraction of a percent. Relative to the controls, hatch rate ranged from about 3% of the control for the shock that started at 59 min after activation to about 22% of the control for the shock that started at 69 min in the Ghana × blond crosses and to about 7% in the red × Ghana crosses (Tables 2 and 3). Increased mortality resulting from the UV-dose was expected due to cell damage. The late-shock protocol for tetraploid induction that was successfully developed by Don and Avtalion (1988a, 1988b) and Don (1989) was used as the basis for induction of androgenesis. Cold shock has a wider time of application (60 min) compared to heat or pressure shock (2 to 5 min) and intuitively should provide a greater likelihood of some successful diploidization. Further, the shock application time used by the Israeli scientists is based on cytological correlations that relate well to time factors as described by tau adjustments (Saat, 1993; Shirak, 1996; Shirak et al., 1998). This is in sharp contrast to the empirical approach of Mair (1993), Hussain et al. (1993) and Myers et al. (1995), where shock time application had little relationship to cytological events.

Hatch in control groups for all six of the R × G crosses and in eleven trials (G × Bl) indicated reasonably good quality ova (Table 2). Induction of androgenetic progeny (UV-treated Ghana ova activated with sperm from blond males or UV-treated ova from red females and activated with sperm from Ghana males) was tested by cold-shocking at three post-activation times (59, 69, and 79 min at 36°C), which correspond to 0.75, 0.87, and 1.0T (T = time of first mitosis) or 1.8, 2.1, and $2.5\tau_0$, respectively. The former (0.75T) is the approximate time for first mitotic metaphase (Saat, 1993; Rubinshtein et al., 1997), while the second (0.87T) is just prior to first mitotic cytokinesis and the optimal induction time for late cold-shock in tilapia as determined by Shirak et al. (1998). The highest hatch rate of androgenotes (non-pigmented larvae) was at the 69-min post-activation shock time; the other two shock times had lower yields. Induction of androgenotes was attempted using eggs from red females and sperm from Ghana males, which were shocked only at $2.1\tau_0$ based on the earlier trials with G × Bl; induction success was generally lower at about 11% compared to about 40% (note, relative to control hatch) in the G × Bl crosses (Tables 2 and 3). Induction success for androgenotes to hatching does not ensure survival to later stages. From a total of 157 androgenotes that hatched from the G × Bl crosses, only 57 survived to swim-up, or yolk absorption (Table 2). Addi-

tional mortality during the juvenile stages further reduces the number that will be available for progeny testing to verify the production of YY males. None of the androgenotes of the G × Bl or R × G crosses survived to maturity, so no verification of sex determination in androgenotes was accomplished.

Optimization of shock protocol is affected by the asynchrony in cell division; at 28°C, the time from initiation of first cleavage to end of the last encompasses about 20 min for Nile tilapia (Shirak et al., 1998). Thus, the shock application and duration will affect only a small percentage of the cells at an optimal induction time. Further, even adjusting for the incubation temperature effect, optimization of treatment time for shock is complicated by apparent differences in effectiveness of various types of shock. Thermal shocks were used in the present study for practical reasons and because their effectiveness has been as good or better than pressure shock. Don and Avtalion (1988a) used cold shock for tetraploid induction based on comparative effectiveness for triploid induction (Don and Avtalion, 1988b). However, the timing for shock reported by Myers et al. (1995) at 27 min post-activation (28°C) markedly contradicts the optimal shock time of 92 min post-activation (26°C) reported by Don and Avtalion (1988a) and Don (1989), even when adjusted for the different incubation temperatures. We reported induction times in the context of developmental rates (τ_0) to adjust for temperature-induced differences. Further, significant differences in optimal shock initiation induction times for tilapia are apparently related to the type of shock (cold vs. heat or thermal vs. pressure). Palti et al. (1997) clearly demonstrated a difference in optimal shock time for gynogenetic induction with heat and pressure shock for rainbow trout, *Oncorhynchus mykiss*. Similarly, Hussain (1995) demonstrated differential optima between cold, heat, and pressure shock in Nile tilapia. Shirak et al. (1998) suggested that cold shock interferes with cell division, while the mechanism for heat shock has been considered to be disruption of the spindle fibers during karyokinesis (Mair, 1993).

Several factors contribute to the low yield and survival of androgenotes. Some of the effects are directly related to the severity of treatment (UV dose and temperature shock) as well as probable genetic influences. Ploidy manipulation depends on the exposure of gametes to near lethal conditions; consequently, elevated mortality is expected. Induced androgenesis includes several critical events: handling, UV exposure, and temperature shock. Eggs are more sensitive to handling than spermatozoa,

Table 3. Induction of androgenotes of Nile tilapia with UV-treated eggs from red tilapia (600 J m⁻²) inseminated with sperm from Ghana strain tilapia and diploidized by cold shock (11 ± 0.5°C for 60 min) at $2.1\tau_0$.

Trial	Number of Eggs*	Number Hatched		Androgenote (Swim-Up)	Survival to Maturity
		Control	*Treatment*		
1	750	40	4	4	0
2	600	28	0	0	0
3	550	42	2	2	0
4	700	22	0	0	0
5	500	36	6	5***	0
6	450	16	0	0	0
Total	3,550	184 (10%)	12 (7%)**	11 (6%)**	0

* Divided between control and treatment; note androgenotes are normally pigmented.
** Relative to control.
*** One red-pigment individual (normally fertilized).

and the higher UV dose contributes to reduced viability; the treated eggs are subsequently subjected to a severe temperature. Because zygotes are diploidized by late shock, all loci are homozygous, which results in an abrupt increase in the pairing of detrimental or lethal genes. Thus, zygotes that survive the trauma of treatment have a reduced viability because of genomic influence.

Finally, evidence is increasing to suggest that sex determination among tilapias is not exclusively controlled by sex chromosomes, i.e., monofactoral, and that Mendelian, and therefore sex, ratios cannot be uniformly expected (Lester et al., 1989; Wolfarth and Wedekind, 1991; Trompka and Avtalion, 1993; Müller-Belecke and Hörstgen-Schwark, 1995). Shelton et al. (1983) reported unexpected variations in sex ratios among progeny of pair-spawned Nile and blue tilapia; Tuan et al. (1999) repeated the pair-spawning for a different strain of Nile tilapia and verified the results of Shelton et al. (1983). Further, Phelps et al. (1999) compared progeny sex ratios from pair-spawns of the three races of Nile tilapia used in the present study and found departures from the expected 1:1.

Poor survival of androgenotes from each broodstock continued throughout the study; the low probability of producing mature YY-males and the increasing evidence of complications with reference to sex determination for tilapias convinced us to suspend the study. Funds for the second year of this study were reallocated in a subcontract to cover a partial stipend for an Auburn University graduate student and travel support for an overseas principal investigator to the annual meeting.

ANTICIPATED BENEFITS

Control of unwanted reproduction in tilapia culture has been one of the primary considerations affecting successful food production. Various approaches have been developed and utilized. Most recently efforts to develop systems that will allow breeding programs to produce monosex cultures have been sought. Androgenesis seemed to offer a mechanism that could directly produce YY-males for use as broodstock to breed all-male progeny. Development of the induction technology was successful, but for various reasons survival of andro-genotes to maturity failed, as did the test of the theoretical basis for sex determination and monosex production from androgenote broodstock.

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