Artificial Propagation of Nile Tilapia for Chromosome Manipulation

Eighth Work Plan, Reproduction Control Research 1B (RCR1B)

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Introduction

Manipulation of fish reproduction, an important scientific development for aquaculture, may be performed at various levels of artificial propagation. Spawning may be controlled through environmental manipulation or through direct hormonal intervention (Shelton, 1989). The basic techniques and capacity for control vary between species. Tilapias do not respond well to hormonal induction but can be induced to spawn through manipulation of environmental variables. A suitable temperature and controlled photoperiod permit a reasonably close prediction of ovulation time.

Chromosome manipulation, which includes ploidy alteration or euploidy induction with single parent genome contribution, requires the collection of freshly released gametes. Ploidy manipulation includes the induction of triploids through polar body retention or tetraploidy through interference with first karyokinesis. Euploid alterations include gynogenetic (gynogenote) diploidization of the maternal genome through polar body retention (meiogynote) or mitotic interference (mitogynote). Diploidization of the paternal genome (androgenote), or androgenesis, is accomplished by interference with first mitosis. In the case of androgenesis and gynogenesis, either the female or male DNA, respectively, must be rendered inactive before the egg is activated. Both parental genomes are intact, however, during ploidy manipulation. This report describes initial efforts in the collection of freshly ovulated eggs from Nile tilapia (*Oreochromis niloticus*) for use in experiments to develop techniques for induction of androgenotes.

Chromosome manipulation involves one or two basic treatments of freshly obtained gametes (Thorgaard and Allen, 1986). For gynogenesis or androgenesis the first treatment is the genome deactivation of the spermatozoa or eggs, respectively. Ultraviolet irradiation is preferred for simplicity and safety, but also because it dimerizes the DNA rather than fragmenting it. Activation of treated spermatozoa or eggs requires diploidization by some form of shock that either retains the second polar body (pb) or interrupts the first mitotic karyokinesis. 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 the simplicity of the equipment used. To disrupt chromosome

separation the shock must coincide with metaphase and be sufficiently severe to disrupt the spindle fibers. Thus, shock intensity, duration, and time of application must be optimally combined to ensure a maximum yield of progeny. Further, because the rate of development is inversely temperature dependent, either the preshock incubation temperature must be standardized or the shock time must be calibrated to compensate for the temperature effect. Absolute shock time (τ_S, in minutes post-activation) can be related to tau (τ_0, in minutes) to report shock protocol in a dimensionless term (τ_S/τ_0) which is temperature compensated (Dettlaff and Dettlaff, 196l; Dettlaff 1986).

The development of a tau curve over the spawning temperature range of Nile tilapia was one of the initial segments of Reproduction Control Research 1 in the Eighth Work Plan.

Methods and Materials

Spawning

This study used the University of Oklahoma stock of *O. niloticus*, obtained from Auburn University, originated in the Ivory Coast, and hybridized to an unknown level with the Egyptian Lake Manzala stock carrying the blond gene (McAndrew et al., 1988). Spawning was managed by photoperiod manipulation. Four to six females were stocked with one male in four 550-l aquaria. Water was aerated and circulated at the rate of one turnover per day and temperature was maintained at 26 ± 2˚C. The light cycle was initiated at 0100 h; the time of sunset signaled the end of the photoperiod for a total of about 20 light hours and 4 dark hours (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 determined through observation of courtship behavior, coloration, and papilla erection (Rothbard and Pruginin, 1975; Rothbard, 1979). Females were stripped after the initiation of spawning or upon observation of other determinants.

Fertilization

Eggs were collected in a clean container, milt was expressed over the eggs, and water was directly added to initiate activation. Within two to three minutes of activation fertilized eggs were placed in controlled temperature (± 0.2˚C) incubators. The incubation temperature was closely regulated to document the embryonic rate of development, so that timing of diploidization shock could be determined. Eggs were incubated in 1-l upwelling units with sufficient flow to gently tumble the developing embryos.

Tau Estimates

Developmental rate is defined as the duration of one mitotic cycle during early synchronous cleavage (Dettlaff and Dettlaff, 1961). The mean interval between the initiation of the first and third mitoses in 5 to 10% of the eggs was recorded at temperatures within the usual developmental range (20 to 30˚C). Twenty to thirty eggs were examined under magnification at five-minute intervals. Tau curves have been used to facilitate chromosome manipulation studies in various fishes (Shelton and Rothbard, 1993; Shelton et al., 1997). The mechanics of this technique have been more difficult with tilapias due to the difficulties associated with gamete collection.

Results

Photoperiod manipulation induced spawning activity to occur at midday. Females ovulated/spawned between 9.5 and 13.5 h (mean = 10.1 h) after initiation of the light cycle. There was no apparent correlation between the latent period and water temperature; however, temperature variation was low (23.9 to 27.7˚C). During the spring 1997 spawning season, eggs were collected from ten Nile tilapia.

Fertilization rates were variable with no development in five of ten batches. Fertilization and hatching rates are poor among tilapias even though egg resorption is common. Egg resorption does not always prevent ovulation of the bad eggs (Peters, 1983). The low rate of fertilization may also be due to the type of incubation system used. The hatch rate of an upwelling type of incubator is not as good as a down-flow system incubator (Rana, 1986). We are in the process of modifying our incubators.

Estimates of tau were made at five temperatures ranging from 20.6 to 27.5˚C (Table 1). The sample size of this study is too small to evaluate variability, however, the estimates of variability have been tightly associated with a tau curve calculated from data on time to first mitosis in *O. aureus* (Don, 1989). The theoretical tau curve for Nile tilapia used in

Table 1. Temperature-tau relationship for Nile tilapia.

Incubation Temperature (°C)	Observed τ_0 (minutes)	Calculated τ_0 (minutes)[1]
20.6	73.5	72.0
21.6	67.5	65.0
22.4	56.5	59.0
24.7	50.0	47.0
27.5	30.0	36.0

[1] Calculated curve developed from data for time to first mitoses in *O. aureus* (Don, 1989) based on methods of Rubenshtein et al. (1997); the calculated tau curve is characterized by the relationship: $\tau_0 = 10^5 x^{-2.39}$ where x = temperature (°C).

this study was generated using the techniques of Rubinshtein et al. (1997). This curve is characterized by the relationship:

$$\tau_0 = 10^5 x^{-2.39}$$

where x is temperature in °C. The tau-temperature relationship showed an inverse correlation and ranged from 73.5 to 30 minutes in the observed temperature range.

Discussion

Ovulation based on the light cycle was a reasonable means for predicting time of stripping. Hormonal induction of ovulation would seem to be a logical method for determination of egg collection; however, cichlids have responded poorly to gonadotropic therapy (Rana, 1988). On the other hand, the physiological characteristics of tilapia gametes provide an advantage in chromosome manipulation. Eggs retain high fertility for three to six hours post-immersion in water (Myers et al., 1995) and sperm remain motile in water for several hours in contrast with most fishes (Yeheskel and Avtalion, 1986). Thus, the quality of gametes should not deteriorate during UV treatment in comparison with other fishes.

More suitable incubation techniques must be incorporated in the chromosome manipulation protocol, since survival is lower for genome-manipulated progeny (Rana, 1986; Mair, 1993). Down-flow incubators should be evaluated in future work.

A reasonable first estimate of a tau curve was developed for Nile tilapia used in this study but additional data should be incorporated. The working curve can be used to standardize shock treatments. The next aspect of this research will be the development of UV treatment of eggs. Treatment of sperm with UV has been routine for the induction of gynogenesis but few studies have attempted androgenesis, which requires egg treatment. Even fewer studies have used UV in female genome deactivation. Preliminary trials have verified the general dosage level (400 to 600 J m^{-2}) reported by Myers et al. (1995). Additional trials will be conducted before attempting diploidization experiments. Finally, shock treatment protocol will be the next research priority. While some guidance is available in the literature for late shock to induce tetraploidy and mitotic gynogenesis (Hussain et al., 1993; Don and Avtalion, 1988a; Mair; 1993), only one study has developed androgenetic protocol for tilapia (Myers et al., 1995).

Thermal shock treatments will be used for practical reasons and because their effectiveness is comparable to or better than pressure shock. Don and Avtalion (1988a) used a cold shock treatment for tetraploidy induction based on its comparative effectiveness for triploidy induction (Don and Avtalion, 1988b). However, the timing for shock reported by Myers et al. (1995) at 27 minutes post-activation (28°C) contradicts the optimal shock time of 92 minutes post-activation (26°C) reported by Don (1989) and Shirak (1986), even when adjusted for temperature. Therefore, these reported optimal times and shock types will be examined within the context of tau information.

A visual genetic marker is vital in chromosome manipulation studies. One option with Nile tilapia is the use of normal-color females and males of the blond mutant (McAndrew et al., 1988). This homozygous recessive trait would usually be appropriate; however, the Ivory Coast stock of Nile tilapia at the University of Oklahoma has been subject to some introgression of the blond gene. Alternatively, the red mutant, which is a dominant gene (McAndrew et al., 1988), could be used for an egg source and normal-color males as sperm donors. I will obtain verified homozygous red mutant stocks from Israel. While developing the red mutant line, androgenetic experimentation shall proceed using the current Nile stock and a gold mutant of *O. mossambicus* (Tave et al., 1989) as the sperm donor.

Anticipated Benefits

The development of androgenesis for *O. niloticus* should provide an alternative method for producing YY-males for the monosex production of tilapia.

Literature Cited

Dettlaff, T.A., 1986. The rate of development in poikilothermic animals calculated in astronomical and relative time units. J. Therm. Biol., 11:1-7.

Dettlaff, T.A. and A.A. Dettlaff, 1961. On relative dimensionless characteristics of the development duration in embryology. Arch. Biol., 72:1-16.

Don, J., 1989. Study of ploidy and artificial induction of gynogenesis in tilapias. Ph.D. dissertation (in Hebrew), Bar-Ilan University, Tel-Aviv, Israel.

Don, J. and R.R. Avtalion, 1988a. Production of viable tetraploid tilapia using the cold shock technique. Bamidgeh, 40:17-21.

Don, J. and R.R. Avtalion, 1988b. Comparative study on the induction of triploidy in tilapia using cold- and heat-shock technique. J. Fish. Biol., 32:665-672.

Hussain, M.G., D.J. Penman, B.J. McAndrew, and R. Johnstone, 1993. Supression of the first cleavage in the Nile tilapia, *Oreochromis niloticus*, L.—a comparison of the relative effectiveness of pressure and heat shock. Aquaculture, 111:263-270.

Mair, G.C., 1993. Chromosome set manipulation in tilapia—techniques, problems and prospects. Aquaculture, 111:227-244.

McAndrew, B.J., F.R. Roubal, R.J. Roberts, A.M. Bullock, and M. McEwen, 1988. The genetics and histology of red, blond and associated colour variants in *Oreochromis niloticus*. Genetica, 76:127-137.

Myers, J.M. and W.K. Hershberger, 1991. Artificial spawning of tilapia eggs. J. World Aquacult. Soc., 22:77-82.

Myers, J.M., D.J. Penman, Y. Basavaraju, S.F. Powell, P. Baoprasertkul, K.J. Rana, N. Bromage, and B.J. McAndrew, 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (*Oreochromis niloticus* L.). Theor. Appl. Genet., 90:205-210.

Peters, H.M., 1983. Fecundity, egg weight and oocyte development in tilapias (Cichlidae, Teleostei). ICLARM Transl. no. 2 (D. Pauly), Manila, Philippines. (Original Publication: 1963, Int. Rev. Ges. Hydrobiol., 48:547-576).

Rana, K.J., 1986. An evaluation of two types of containers for the artificial incubation of *Oreochromis* eggs. Aquacult. Fish. Mgt., 17:139-145.

Rana, K.J., 1988. Reproductive biology and the hatchery rearing of tilapia eggs and fry. In: J.F. Muir and R.J. Roberts (Editors), Recent Advances in Aquaculture, 3. Croom Helm, London, pp. 343-406.

Rothbard, S., 1979. Observation on the reproductive behavior of *Tilapia zillii* and several *Sarotherodon spp*. under aquarium conditions. Bamidgeh, 31:35-43.

Rothbard, S. and Y. Pruginin, 1975. Induced spawning and artificial incubation of tilapia. Aquaculture, 5:315-321.

Rubinstein, I., S. Rothbard, and W.L. Shelton, 1997. The relationship between the embryological age, cytokinesis-1 and the timing of ploidy manipulations in fish. Israeli J. Aquacult. - Bamidgeh 49:99-110.

Shelton, W.L., 1989. Management of finfish reproduction for aquaculture. CRC Rev. Aquat. Sci., 1:497-535.

Shelton, W.L. and S. Rothbard, 1993. Determination of the developmental duration (τ_0) for ploidy manipulation in carps. Israeli J. Aquacult. - Bamidgeh, 45:73-81.

Shelton, W.L., S.D. Mims, J.A. Clark, A.E. Hiott, and C. Wang, 1997. A temperature-dependent index of mitotic interval (τ_0) for chromosome manipulation in paddlefish and shovelnose sturgeon. Prog. Fish-Cult., 59:229-234.

Shirak, A., 1996. Chromosome set manipulations in tilapias: a model of lethal gene inheritance. M.S. thesis (in Hebrew), Bar-Ilan University, Tel Aviv, Israel.

Tave, D., M. Rezk, and R.O. Smitherman, 1989. Genetics of body color in *Tilapia mossambica.* J. World Aquacult. Soc., 20:214-222.

Thorgaard, G.H. and S.K. Allen, Jr., 1986. Chromosome manipulation and markers in fishery management. In: N. Ryman and F. Utter (Editors), Population Genetics and Fishery Management. Washington Sea Grant Program, University of Washington Press, Seattle, pp. 319-331.

Yeheskel, O. and R.R. Avtalion, 1986. Artificial fertilization of tilapia eggs, a preliminary study. In: Reproduction in Fish—Basic and Applied Aspects in Endocrinology and Genetics. Les Colloques de l'INRA no. 44, Institut National de la Recherche Agronomique, Paris, pp. 169-175.