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Induced Spermiation in the Male Spotted Scat *(Scatophagus argus)* by Long-Term Administration of 17α-Methyltestosterone Followed by LHRHa

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Abstract

Studies were undertaken to find a reliable method to induce spermiation in male apotted scat (Scatophagus argus), a herbivorous, eu,yhalline teleast which can be readily reared in tropical brackish water fish ponds. Administration of HCG (5 and 10 IU1(1), LHRHa ar l 7a-methyltestosterone (MT) alone, or LHRHa pluo MT administered simultaneously, were ineffective treatments for stimulating spermiation. Prolonged administration of MT (15 or 45 days), followed by the administration of LHRHa in a cholesterol-based pelleted implant (35 mg-kg-1), however, aignificantly otim,,lated apermiation.

Introduction

The spotted scat, *Scatophagus argus,* is one of few teleost species of economic importance that can thrive in tropical brackishwater fish ponds (Bardach et al. 1972; Fast et al. 1989). It is a preferred food fish in many parts of Southern and Southeast Asia, and in the Philippines,

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is considered a delicacy. Scats are also important in the tropical aquarium fish trade. Fast et al. (1989) concluded that the scat's slow growth rate will probably preclude the development of an economically viable growout system for the food market. On the other hand, the pond culture of spotted scat has good potential to meet the needs of the aquarium fish trade. To achieve this however, technologies for induced breeding and larviculture of the scat must be developed since the seasonally variable supply of wildcaught scat fry cannot meet the current high demand for this species (Bon Eng Ty, pers. comm.).

In our preliminary spawning trials, we easily induced females to spawn using implants of LHRHa, but did not achieve fertilization because we were unable to obtain sufficient amounts of milt from the males. Studies were conducted, therefore, to develop a reliable methodology for inducing spermiation in male scats.

Materials and Methods

Experimental Animals

Adult male spotted scat were collected from the southern coastal waters of Panay Island, Philippines. On the day following capture, the fish were individually weighed (±0.1 g) and measured (standard length ±0.1 cm) under anesthesia (0.3 ml/l 2-phenoxyethanol). Male scat reach sexual maturity at approximately 12 cm in standard length (Barry and Fast 1988). Fish this size and larger were chosen for the experiments and individually tagged. Male fish were sampled for running milt by gently squeezing their abdomens, and were classified according to the following scale: (0) = no milt; (+) = small volume of milt after repeated squeezes; (++) = large volume of milt after a single squeeze. The fish were stocked in hapa nets or floating cages in earthen ponds, or held in 1-tonne (1 m^3) concrete, or 9-tonne (9 m^3) canvas tanks. The fish were fed commercial shrimp or milkfish pelleted feeds containing 35 and 27% crude protein, respectively. This diet was supplemented with filamentous and benthic algae *(lab-lab)*.

Hormone Administration

Luteinizing hormone-releasing hormone analogue, des-Gly^{10}, [D-Ala^6] LHRH (LHRHa), 17α-methyltestosterone (MT) and cholesterol

were purchased from Sigma Chemical Co., St. Louis, MO, USA. Human chorionic gonadotropin (hCG) was purchased from the Argent Chemical Co., Redmond, Wa. Cholesterol-based pellets of LHRHa were made according to the method described by Lee et al. (1985). Each pellet contained 12.2 mg of cholesterol and 0.64 mg of melted cocoa butter, and was approximately 8.1 mg in weight, 4.0 mm in length and 1.5 mm in diameter. LHRHa pellets were implanted into an incision made in the dorsal musculature.

Methyltestosterone was administered in one of three ways: 1) Silastic tubes were prepared according to the method described by Lee et al. (1985). A small incision was made in the abdominal wall and the silastic tube containing MT dissolved in castor oil was implanted into the peritoneal cavity. One or two sutures were made to close the incision; 2) Using the method described by Higgs et al. (1977), commercial milkfish pellets were sprayed with MT dissolved in 95% ethanol to produce feed containing 10 mg MT per kg of feed. The experimental fish were fed the steroid-containing feed at a rate of 5% body weight per day; control fish received feed sprayed with ethanol; and 3) Crystalline MT was incorporated into cholesterol-based pellets and implanted into the dorsal musculature.

Experimental Designs

EXPERIMENT 1

There were four treatment groups: 1) control, 2) 10 μg LHRHa, 3) 1 mg MT, and 4) 1 mg MT plus 10 μg LHRHa. Each group contained four fish. All treatments were administered in cholesterol-based pellets. The fish weighed 100-450 g (average 270 ± 24 g), and therefore, the hormone doses varied from 22 to 100 $\mu g \cdot kg^{-1}$ for LHRHa, and from 2.2 to 10 $mg \cdot kg^{-1}$ for MT. Fish were sampled daily for milt production.

EXPERIMENT 2

The effects of hCG were evaluated in groups of fish injected with 5 or 10 IU/g. There were three fish per group. One-third of the full hormone dose was administered intramuscularly in the late afternoon, and the remaining two-thirds 12 hours later. Control fish received saline only.

EXPERIMENT 3

Six fish were implanted with pellets containing 25 μg LHRHa; five received control pellets. Fish weighed 176-375 g (average weight = 271 ± 32 g); therefore, the doses of LHRHa varied from 66 to 142 $\mu g \cdot kg^{-1}$. The fish were monitored every 8 hours.

EXPERIMENT 4

On September 15, four males were placed into each of the following treatment groups: 1) control, 2) MT-fed (10 $mg \cdot kg^{-1}$ feed), and 3) MT silastic implant (250 μg MT). On October 16, two fish each were added to groups 1 and 2.

On October 30, 45 days into the experiment, all the fish from the MT-fed and control groups were implanted with the same dose of LHRHa (pellets containing 35 $\mu g \cdot kg^{-1}$). Only two fish from the MT implant group survived until October 30. One was administered 35 $\mu g \cdot kg^{-1}$ LHRHa, and the other received a control pellet. The fish were sampled for milt on the dates indicated in Table 1.

STATISTICAL ANALYSIS

At each sampling time, an individual was recorded as being either with (+ or ++) or without (-) expressible milt. The total number of sampling dates when the fish within a given treatment group were found with milt was divided by the total number of sampling dates. The value obtained (% milting) gives the percentage of fish milting over the course of the experiment. To calculate the % milting value, + and ++ fish were not differentiated. An R x C Test of Independence using the G-Test (Sokal and Rohlf 1981) was used to test the null hypothesis that milting response was independent of the hormone treatment.

Results

The % milting values for the fish in the four treatment groups of experiment 1 were as follows: control 36.4%; 10 μg LHRHa 38.1%; 1 mg MT 14.3%, and LHRHa plus MT administered simultaneously 25.0%. There were no significant differences among these treatments.

Treatment of male scat with hCG actually decreased the percentage of fish with expressible milt relative to controls. The differences, however, were not significant at the 5% level. In experiment 2, the %

milting values were as follows: control 33.3%; 5 IU/g body weight hCG 17.5%, and 10 IU/g body weight hCG, 21.4%

In experiment 3, LHRHa implants of 25 μg per fish resulted in a 63.9% spermiation response compared with 50.0% for controls. This difference, however, was not significant.

In experiment 4, spermiation was not significantly enhanced relative to the controls in either the MT-fed or MT-implanted fish between September 15 and October 30 (Table 1). One control and one MT-implanted fish were spermiating on October 16, but neither was spermiating when next sampled on October 29. Two MT-implanted fish died before October 16.

Following the implantation of LHRHa on October 30, there was a dramatic increase in the number of milting males. One control and two MT-fed fish responded to LHRHa within 24 hours. Within 48 hours, four of the five MT-fed males had expressible milt. Three of these four fish were still producing measurable amounts of expressible milt 24 days later, on November 23, when the experiment was terminated. In contrast, not a single control fish was found milting 48 hours after LHRHa implementation. One male from the control group was found milting from November 8 to 23. In this fish, an 8-day latency period followed LHRHa implantation before it began to produce milt. Following implantation of LHRHa, the % milting values for the control and MT-fed fish were 18.2 and 61.8%, respectively. This difference was highly significant ($P < 0.001$).

In the MT-implanted group, the male that received LHRHa began milting four days following implantation; the control fish showed no change (Table 1).

In summary, MT alone had no effect on milt production in male scat (Table 1). When 35 $\mu g \cdot kg^{-1}$ of LHRHa was administered to MT-fed or MT-implanted fish, however, there was a highly significant spermiation response. LHRHa had little or no effect on males not previously exposed to MT.

Discussion

Unlike the situation in various other teleosts (Harvey and Hoar 1979; cf. Donaldson and Hunter 1983; Weber and Lee 1985), hCG, MT or LHRHa alone could not induce spermiation in male spotted scat. LHRHa was highly effective, however, when administered to MT-primed fish. There was clearly some interaction between these two hormones delivered in sequence that produced a much greater spermiation

Table 1. Induced spermiation in male *Scatophagus argus*. Effects of MT followed by LHRHa.

		Sampling date and spermiation response[1]																
		Before LHRHa						After LHRHa										
Treatment[2]	Fish	Sep 15	18	Oct 02	16	29	30	31	Nov 02	03	04	08	10	13	16	18	20	23
	1	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-
Control	3	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-
	5				++	-	L	+	-	+	-	-	-	+	+	+	-	-
	6				-	-	L	-	-	-	-	+	+	+	+	+	+	+
	7	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-
MT fed	8	-	-	-	-	-	L	+	+	+	+	+	-	+	+	+	+	+
	9	-	-	-	-	-	L	-	+	++	-	+	+	+	+	+	+	+
	10				-	-	L	-	+	++	-	+	+	+	+	+	+	+
	11				++	-	L	+	+	+	+	+	-	+	-	-	-	-
	12	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-
MT implant	13	-	-	-	-	-	L	-	-	+	+	+	+	+	+	+	+	+
	15	-	-		died													
	16	-	-	-	died													

[1](-) no milt; (+) some milt; (++) greater amounts of milt than (+).

[2]Beginning 15 September 1987, male *Scatophagus argus* were fed commercial pellets treated with (MT fed) or without (control) 17α-methyltestosterone (10 mg·kg^{-1} of feed at a rate of 5% of their body weight per day). Other males were implanted with silastic tubes containing 250 mg MT (MT implant). On 30 October 1987, fish were implanted with cholesterol-based pellets containing 35 mg·kg^{-1} LHRHa (L), or given a control pellet (C).

response than administration of either hormone alone or both hormones delivered simultaneously.

The effects of LHRHa were almost certainly mediated through its ability to stimulate gonadotropin (GtH) release from the pituitary; GtH then acted on the testes to induce spermiation (Peter 1980; Donaldson and Hunter 1983; Van der Kraak et al. 1983; Fostier et al. 1983; Billard et al. 1984). Several of the MT-primed fish continued to spermiate for over three weeks following LHRHa implantation, suggesting that our cholesterol-based pellets continued to release LHRHa for at least that long in agreement with previously published data (Weil and Crim 1982; Crim et al. 1983; Crim 1985; Lee et al. 1986; Nacario and Sherwood 1986; Crim et al. 1987; Almendras et al. 1988; Sherwood et al. 1988).

Some hypotheses about the role of MT, and possible reasons why only the MT-primed fish responded to LHRHa, are that MT: 1) stimulated the accumulation of GtH in the pituitary (Crim and Evans 1979); 2) regulated the production and maturation of spermatozoa, i.e., stimulated spermatogenesis (Fostier et al. 1983); 3) somehow modulated the effects of GtH on the testes; or 4) enhanced the effects of LHRHa on the pituitary (Fostier et al. 1983). The first two hypotheses may be more likely since both of these processes would require a long time and can explain why the acute administration of MT with LHRHa had no effect in inducing spermiation (Experiment 1), whereas chronic administration of the steroid prior to LHRHa implantation was effective.

Regardless of the mechanism of action, prolonged treatment with MT followed by implantation of LHRHa in a cholesterol-based pellet is an effective method for inducing spermiation in *S. argus*. It remains to be investigated which doses of MT and LHRHa will give an optimal spermiation response, and whether shorter periods of MT administration will also prove effective.

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References

Almendras, J.M., C. Dueñas, J. Nacario, M.N. Sherwood and L.W. Crim. 1988. Sustained hormone release. III. Use of gonadotropin releasing hormone analogues to induce multiple spawnings in seabass, *Lates calcarifer*. Aquaculture 74: 97-111.

Bardach, J.E., J.H. Ryther and W.O. McLarney. 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley, New York.

Barry, T.P. and A.W. Fast. 1988. Natural history of the spotted scat *(Scatophagus argus)*, p. 4-30. *In* A.W. Fast (ed.) Spawning induction and pond culture of the spotted scat (*Scatophagus argus* Linnaeus) in the Philippines. Mariculture Research and Training Center, Hawaii Institute of Marine Biology, University of Hawaii at Manoa.

Billard, R., K. Bieniarz, R.E. Peter, M. Sokolowka, C. Weil and L.W. Crim. 1984. Effects of LHRH and LHRH-A on plasma GtH levels and maturation/ovulation in the common carp, *Cyprinus carpio*, kept under various environmental conditions. Aquaculture 41: 245-254.

Crim, L.W. 1985. Methods for acute and chronic hormone adminiatration in fish, p. 1-13. *In* C.S. Lee and I.C. Liao (eds.) Reproduction and culture of milkfish. Oceanic Institute and Tungkang Marine Laboratory, 22-24 April 1985. Tungkang Marine Laboratory, Taiwan.

Crim, L.W. and D.M. Evans. 1979. Stimulation of pituitary gonadotropin by testosterone in juvenile rainbow trout *(Salmo gairdneri)*. Gen. Comp. Endocrinol. 37: 192-196.

Crim, L.W., N.M. Sherwood and C.E. Wilson. 1987. Sustained hormone release. II. Effectiveness of LHRH analogue (LHRHa) administered by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74: 87-95.

Crim, L.W., A.M. Sutterlin, D.E. Evans and C. Weil. 1983. Accelerated ovulation by pelleted LHRH analogues treatment by spring-spawning rainbow trout *(Salmo gairdneri)* held at low temperature. Aquaculture 35: 299-307.

Donaldson, E. and G. Hunter. 1983. Induced final maturation, ovulation and spermiation in cultured fish, p. 351-403. *In* W.S. Hoar, D.J. Randall and E.M. Donaldson (eds.) Fish physiology, Vol. IXB, Academic Press, New York.

Fast, A.W., H. Biona, R. Tabanda, R. Bayogos and T. Barry. 1989. Brackishwater pond culture of the spotted scat *(Scatophagus argus)* in the Philippines. J. Aquacult. Trop. 4: 37-49.

Fostier, A., B. Jalabert, R. Billard, B. Breton and Y. Zohar. 1983. The gonadal steroids, p. 277-371. *In* W.S. Hoar, D.J. Randall and E.M. Donaldson (eds.) Fish physiology, Vol. IXA, Academic Press, New York.

Harvey, B.J. and W.S. Hoar. 1979. The theory and practice of induced breeding in fish. IDRC, Ottawa, Ontario. p. 7-41.

Higgs, D.A., U.H.M. Fagerlund, J.R. McBride, H.M. Dye and E.M. Donaldson. 1977. Influence of combination of bovine growth hormone, 17α-methyltestosterone and L-thyroxine on growth of yearling coho salmon *(Oncorhynchus kisutch)*. Can. J. Zool. 55: 1048-1056.

Lee, C.S., C.S. Tamaru, J.E. Banno, C.D. Kelly, A. Bocek and J.A. Wyban. 1986. Induced maturation and spawning of milkfish, *Chanos chanos* Forsskal, by hormone implantation. Aquaculture 52: 199-205.

Lee, C.S., C.S. Tamaru and L.W. Crim. 1985. Preparation of a luteinizing hormone releasing hormone cholesterol pellet and its implantation in the milkfish (*Chanos chanos* Forsskal), p. 215-226. *In* C.S. Lee and I.C. Liao (eds.) Reproduction and culture of milkfish. Oceanic Institute and Tungkang Marine Laboratory, 22-24 April 1985. Tungkang Marine Laboratory, Taiwan.

Nacario, J.F. and N.M. Sherwood. 1986. The use of LHRH analogues in the spawning of the seabass, *Lates calcarifer* (Bloch): comparison of pellet, injection and osmotic pump, p. 13. *In* The First Asian Fisheries Forum, Scientific Sessions Abstracts, Manila, Philippines.

Peter, R.E. 1980. Serum gonadotropin levels in mature male goldfish in response to luteinizing hormone-releasing hormone (LHRH) and des-Gly^{10}-[D-Ala^{6}]-LHRH ethylamide. Can. J. Zool. 58: 1,100-1,104.

Sherwood, N.M., L.W. Crim, J. Carolsfeld and S.M. Walters. 1988. Sustained hormone release. I. Characteristics of *in vitro* release of gonadotropin-releasing hormone analogue (GnRH-A) from pellets. Aquaculture 74: 75-86.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd edition. W.H. Freeman and Company, San Francisco, CA, 859 p.

Ty, R.B.E. 1987. President, Aquascapes Philippines Co., Seascapes Development Corp., NAIC Aquaculture Inc., Quezon City, Philippines.

Van der Kraak, G., H.R. Lin, E. Donaldson, H. Dye and G. Hunter. 1983. Effects of LH-RH and des-Gly^{10}(D-Ala^{6}) LH-RH-ethylamide. Plasma gonadotropin levels and oocyte maturation in adult female coho salmon *(Oncorhynchus kisutch)*. Gen. Comp. Endocrinol. 49: 470-476.

Weber, G.M. and C.S. Lee. 1985. Effects of 17α-methyltestosterone on spermatogenesis and spermiation in the grey mullet, *Mugil cephalus* L. J. Fish Biol. 26: 77-84.

Weil, C. and L.W. Crim. 1982. The effects of different methods of administration of LH-RH analogs on spermiation in the mature landlocked salmon, *Salmo salar*. Can. Soc. Zool. Bull. 13(2): 60 (Abstract)

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