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INDUCTION OF MATURITY AND SPONTANEOUS SPAWNING OF CAPTIVE BROODSTOCK OF BHETKI *LATES CALCARIFER* (Bloch) THROUGH HORMONAL MANIPULATION

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Central Institute of Brackishwater Aquaculture (CIBA), Chennai for the first time in India, has succeeded in the development of seed production technology for the priority candidate species sea bass (*Lates calcarifer*). Captive land based broodstock of *Lates calcarifer* has been successfully developed. Broodstock of sea bass held in 100 tonne capacity RCC tanks with proper water, health and feed management strategies could be made to attain gravid condition through LHRH-a hormone treatment. Female fishes weighing 6-10kg size were implanted with LHRH-a hormone pellet intramuscularly. Fishes with average ova diameter of 346μ-391μ were implanted with hormone pellet @ 100μg/kg body weight to accelerate the maturation. Within 45 days of implantation fishes attained gravid condition with mean ova diameter of more than 450μ suitable for induction of spawning. However, fishes without hormone implantation with initial mean ova diameter of 354μ and 355μ attained maturity only after 80 and 109 days respectively showing the importance of hormone manipulation to accelerate and advance the maturity stages. These fishes were induced to spawn through LHRH-a hormone injection @ 60-70μg/kg body weight for females and 30-35μg/kg body weight for males. Spawning was spontaneous after 32-35 h of injection. Fertilization rate was from 40 to 80% with hatching rate between 25-90%. The details are discussed.

INTRODUCTION

Sea bass, popularly known as Bhetki in India is considered as 'Aqua Star' for farming. It is a sturdy and hardy fish capable of withstanding wide environmental fluctuations. Asian Sea bass, *Lates calcarifer* (Bloch) is an important coastal, estuarine and freshwater fish in the tropical Indo-Pacific region (Greenwood, 1976). It is extensively cultured in ponds and cages of coastal and inland water ecosystems. In India, attempts

were made for experimental culture of this species (Anon, 1985). However, Sea bass farming has not picked up on large scale mainly due to the non availability of adequate quantity seed. To ensure a reliable supply of seed at the time of need, suitable hatchery technology has to be developed for large scale seed propagation which would promote sustainability in aquaculture of Sea bass.

Induction of gonadal maturation of captive brood stock of L.calcarifer and successful spawning was first achieved in Thailand by Wongsomnuk and Manevonk (1973). This was followed successfully in the Philippines (Harvey *et al.,* 1985), Taiwan (Lin *et al.,* 1985), Singapore (Lim *et al.,* 1986), Malaysia (Ali, 1987) and in Australia (Mackinnon, 1987). In India, the reproductive physiology and breeding behavior of the wild sea bass have been studied by various workers (Naidu, 1939; Pillay, 1954; Rao, 1964; Jones and Sujansingani, 1954; Jhingran and Natarajan, 1966; Jhingran, 1969; Ghosh, 1973; Patnaik and Jena, 1971; Kowtal, 1977; James and Marichamy, 1986). Captive maturation of brood stock *Lates calcarifer* has been reported by Mathew Abraham *et al.,* (1996).

Realising the need of the development of indigenous technology for the sea bass seed production, Central Institute of Brackishwater Aquaculture (CIBA), Chennai took up sea bass seed production technology development as priority programme. The present investigation on the induction of gonadal maturation of the land based captive brood stock through hormonal manipulation for successful spawning and seed production of *Lates calcarifer* is a part of fish seed production technology programme.

MATERIALS AND METHODS

Broodstock fish-collection, transportation and maintenance under captivity:

Fishes in the size group ranging from 490mm/2.0kg to 860mm/10.0kg were procured during July to September 1995 from Kovalam and Muttukkadu coastal area catches. The fishes were mainly caught using hook and line. Fishes were transported to the hatchery in open container like plastic buckets/tubs filled with sea water. They were kept in a 250l FRP tanks individually and treated with 1.0 ppm acriflavine for 15 minutes as prophylactic treatment to avoid secondary infection due to injury caused by fishing and handling. Fishes were stocked in 750 sq.m earthern pond and maintained in the ambient conditions from July to December 1995. Afterwards, the fishes were transferred to RCC (Reinforced Cement Concrete) tank (12mx6mx2m) with the net water capacity of 100 tonne. The stocking density of brood fishes was maintained in the tank @ 1.0 kg/m^3. Tanks were cleaned daily to remove the debris/faecal matter and water exchange to an extent of 80% with salt water drawn from bore well put up in inter tidal areas was done. Fishes were fed with frozen trash fishes such as *Oreochromis mossambicus* and *Sardinella sp* @ 5% body weight once in a day. Health monitoring was done regularly by examining the gills, scales and fins to assess parasite infection if any. Water quality parameters such as temperature, salinity, pH and dissolved oxygen were monitored once in two days and ammonia and nitrite levels were measured at fortnightly intervals and maintained in the range of 26.5-34.0°C, 26.0-34.0 ppt, 7.9-8.3, 5.5-8.8ppm, 0.002-0.120ppm and 0.002-0.11ppm respectively.

Assessing Sexual Maturity

Gonadal maturity stages of the captive broodstock were examined at fortnightly intervals. The state of ovarian maturity of female fish was assessed by the *in vivo* monitoring method validated by Shehadeh *et al.*, (1973a). One end of Polyethylene cannula, with a diameter of 1.5 mm was inserted through the oviduct to a distance of 10-15 cm and the other end of the cannula was aspirated slowly withdrawing the inserted end. The oocytes were then transferred to a glass slide and measurement was made using calibrated microscope. The males were found to be in running condition and releasing milt when the abdomen was gently pressed.

Implantation of LHRH-a Hormone Pellet

To accelerate/advance the gonadal maturation in fishes hormone pellet implantation was attempted. The male was in oozing stage under normal condition without any manipulation. Control fishes were kept without pellet. This technique involves implantation of LHRH-a hormone incorporated and pelleted in matrix of cholesterol powder (Parazo *et al.*, 1990). Three females were implanted with LHRH-a hormone @ 100μg/kg body weight during first week of June, 1997. These fishes were tagged separately and maintained in the broodstock fish holding tank. The fishes were assessed for ovarian maturation at fortnightly intervals till oocyte attain required size for induction of spawning.

Hormonal Induction of Spawning

Female fishes with mean ova diameter of above 450 μ and males with oozing condition were selected for induced breeding experiments. A total of five experiments

were conducted using LHRH-a hormone. The dose was 60-100μg/kg body weight for females and 30-35 μg/kg body weight for males. Hormone was injected intra muscularly using a hypodermic syringe just below the dorsal fin. Both male and female fishes were kept together in 20 tonne capacity RCC tank (2.5m x 4m x 2m) in the ratio of 2:1 with aeration. Hormone administration was usually done during morning hours 0830 - 1100hrs. Water temperature and salinity in the spawning tank were maintained at 29.0 $\pm$ 0.5°C and 33.0 $\pm$ 1.0 ppt respectively. After spawning, the fertilized eggs were transferred into incubation tanks of 500l capacity for hatching. The percentage of fertilization and hatching rate were estimated.

RESULTS

Results on induction of ovarian development in *Lates calcarifer* using LHRH-a hormone is presented in table 1. The three female fishes which had initial mean ova diameter of 391μ, 363μ and 346μ respectively showed significant advancement in ovarian maturation after 20 to 49 days of LHRH-a hormone pellet implantation @ 100 μg/kg fish body weight. The oocyte diameter increased to a size of 473μ, 458μ and 454μ respectively. However, fishes with initial mean ova diameter of 354μ and 355μ which were not implanted with hormone pellet had the mean oocyte diameter of 398μ and 402μ respectively during the same period. These fishes showed improvement in mean ova diameter of 451μ and 464μ respectively during first week of September '97 and October'97 (after 80 and 109 days). The faster ovarian development of fishes with hormonal treatment than that of fishes without hormonal treatment clearly indicated that the hormone could accelerate and advance the gonadal maturity earlier than normal process of maturation under captive condition.

Induced breeding experiment results in *Lates calcarifer* are presented in table 2. In the first experiment, the female fish in the size of 860mm/10.0 kg which had mean ova diameter of 473μ was administered with LHRH-a hormone @ 100μg/kg body weight. Two male fishes in the same size of 510mm/2.0 kg each were released along with female in the spawning tank without any hormone treatment initially. The response of female was slow upto 24 h and afterwards, the belly enlarged with swollen abdomen indicating ovulation. The males were administered with LHRH-a hormone @ 35μg/kg body weight and released into the spawning tank. However, the fish did not spawn even after 48 h of injection and belly remained swollen with mass of ovary, plugging the genital pore. Then the fish was anaesthetised and stripping was attempted by dry method. After mixing the milt and eggs for four minutes, the eggs were transferred to incubation tank. The eggs were found to be in ovulated condition with a mean ova diameter of 730μ. However, the eggs were opaque and settled at the bottom. No fertilization was observed.

In the second experiment, 820mm/9.0 kg female fish which had an initial mean ova diameter of 458μ was selected for induced breeding. The fish was injected with LHRH-a hormone @ 70μg/kg body weight at 0830 hrs. Two oozing males of 510mm/2.0 kg and 540mm/2.5kg size were also administered with LHRH-a @ 35μg/kg body weight. Both male and female fishes were released in the spawning tank. Spawning response was observed by the closer movement and courtship of both female and males in the tank. Enlargement of belly was observed after 24 h of injection. After 35 h of hormone injection, around 1930 h spontaneous and natural spawning was observed. Spawning activity was associated with awkward and faster movement of the fishes. At the time of spawning, fishy odour could be felt upto few meter distance from the spawning tank. The fertilized eggs were transparent and floating and unfertilized eggs were opaque and settled at the

bottom. Totally, 1.46 million eggs were obtained. The rate of fertilization was 70%. The average diameter of the fertilized eggs was 0.794mm. The fertilized eggs were then transferred into incubation tank for hatching and the hatching took place after 1600 h of fertilization. The hatching rate was 80%. The newly hatched larvae measured an average total length of 1.732 mm. Second spawning of the same fish was observed 24 h after first spawning and 1.30 million eggs could be obtained. However, these eggs were not fertilized.

In the third experiment female fish size was 650mm/6.0 kg with the initial mean ova diameter of 454μ. The fish was administered with LHRH-a @ 60μg/kg body weight at 0830 h. Two oozing males each 490mm/2.0kg and 500mm/2.75kg were also administered with LHRH-a hormone @ 35μg/kg body weight. The fish spawned 35 h after hormone injection and 1.05 million eggs were obtained in the first spawning. After 24 h of first spawning, second spawning occurred and 1.0 million eggs were obtained. Rate of fertilization was estimated about 80% in the first spawning. Fertilization was not successful in the second spawning. The fertilized eggs hatched out 17 h after the fertilization. Hatching rate was 90%.

In the fourth experiment, the size of the female was 790mm/8.0 kg with mean ova diameter of 451μ. Female fish was administered with LHRH-a @ 70μg/1kg body weight at 1000 h. Oozing males of 480mm/2.0kg and 485mm/2.55kg size were also administered with LHRH @ 35μg/kg body weight at the same time. Spawning occurred after 32 h of hormone injection. About 1.20 million eggs were obtained in the first spawning and the rate of fertilization was 70%. Hatching occurred 17 h after fertilization and hatching rate was about 60%. After first spawning, the female fish was transferred into another RCC tank of 20 tonne capacity for second spawning. Fresh set of males with oozing condition

in the size of 490mm/2.0kg and 510mm/2.5kg were administered with LHRH-a @ 30μg/kg body weight and released into spawning tank. After 24 h of first spawning, second spawning was observed and about 0.90 million eggs were obtained. The rate of fertilization was 40% and hatching rate was 25%.

The size of the female fish used in the fifth experiment was 760mm/7.0kg with ova diameter 464μ. The size of the male fishes was 500mm/3.0kg and 480mm/2.5kg. The female was administered with LHRH-a hormone @ 70μg/kg body weight and for males the dosage was @ 30μg/kg body weight. The fishes were released into spawning tank. The spawning was spontaneous and natural from which 1.10 million eggs and 1.00 million eggs were obtained in the first and second spawning respectively. The first spawning was after 32 h of hormone injection and the second spawning of the same set of fishes was 24 h after first spawning. Fertilization rate was 80% in the first spawning and 60% in the second spawning. The hatching rate was 70% in the first spawning and 53% in the second spawning. In all the experiments, during incubation period, water temperature, salinity and pH were maintained at 27.0 $\pm$ 0.5°C, 32.0 $\pm$ 1.0 ppt and 8.15 $\pm$ 0.30 respectively.

From the results obtained from these experiments, it could be observed that the LHRH-a hormone dose ranging between 60 and 70μg/kg body weight for females and 30-35μg/kg body weight for males could successfully induce the spawning of sea bass. A high dose of 100μg/kg body weight has lead to plugging. Successive spawning of *Lates calcarifer* after 24 h of first spawning was noticed. Spawning occurred usually during late evening from 1900 - 2200 h after 32-35 h of hormone injection. Rate of fertilization was in the range of 40 to 80% and hatching rate was between 25 and 90%. Hatching took place between 16 and 17 h after fertilization.

DISCUSSION

The use of pelleted hormone to induce gonadal development and to synchronize ovulation and spawning in a variety of cultured fish species have been gaining considerable popularity (Lam, 1982). The present investigation demonstrated that the implantation of pelleted LHRH-a is effective in stimulating gonadal development in sea bass and has also revealed that the maturation process of sea bass could be accelerated/advanced to required oocyte size (450μ and above) for breeding earlier than the control fish with a dose of 100μg/kg LHRH-a body weight. Crim (1985) and Crim *et al.*, (1987) have stated that LHRH-a embedded in a pelleted powder matrix could be gradually released in to the circulatory system to chronically stimulate the pituitary - gonadal axis for an extended time period and demonstrated the release of gonadotropin hormone (GTH) for several days following *in vivo* single implantation of LHRH-a pellet. The effectiveness of these hormonal therapies for accelerating/advancing the gonadal maturity has been substantiated by other workers also (Nacario, 1987). Garcia (1990) has pointed out that pelleted LHRH-a alone or in combination with methyltestosterone could advance ovarian maturation in sexually immature sea bass as early as normal season when a dose of 200μg/kg body weight was administered.

In the present study, induced breeding of sea bass was successful when the fish was administered with LHRH-a @ 60-70μg/kg body weight for females and 30-35μg/kg body weight for males. However, a high dose of 100μg/kg body weight was found to be not successful to induce the spawning. Garcia (1990) has also observed decline in spawning rate at the two highest doses of LHRH-a (150μg/kg and 300μg/kg body weight) in *Lates calcarifer*. Peter (1980) has observed suppression of serum GTH level in circulatory system after three daily injection of a high dose of super active LHRH-a in mature

gold fish, implying a self-suppressive action by LHRH-a on the GTH releasing mechanism. Garcia (1989a) suggested optimum dose of 38 to 75μg LHRH-a/kg body weight to induce sequential spawning in sea bass. These reports are in agreement with the results obtained in present experiments, where single high dose of LHRH-a 100μg/kg body weight has lead to plugging and the optimum dose was found to be 60-70μg/kg body weight for females and 30-35μg/kg body weight for males. Sea bass spawned during late evening hours in all the experiments, similar observations were made by Maneewong and Watnabe (1984). The critical initial egg size was found to be of 0.4 mm and above in sea bass that represent the stage were the oocyte is both morphologically and physiologically ready to undergo the final stage of maturation and spawning (Garcia, 1989b). In the present study also fishes treated with hormone for spawning were with the ova diameter in the range of 451-473μ from which successful spawning was observed. Garcia (1989a) has opined that although fully-ripe females with initial oocyte diameter of more than 0.5 mm spawn even without hormonal intervention, the use of LHRH-a may still be a highly effective for obtaining eggs on demand from these fully-riped females. In general, the number of eggs spawned on the first day was greater than that on next day. The results obtained in the present experiments agreed well with the report of Almendras *et al.*, (1988), who have also observed similar result when the sea bass was induced for multiple spawning by LHRH-a pellet implantation. However, Suzuki (1983) found no correlation of eggs fertilizability or hatchability with the incidence of multiple spawning in loach.

In the present study, though successive spawning was observed in almost all the cases, fertilization was not effected in two cases, which might be due to non-availability of potential sperm to fertilize the eggs. Since the males used were small in size they could not cop up with the milt requirement. When males were replaced with new sets, successful fertilization was observed. However, in one case, successful fertilization was

observed when the same set of males were kept in the tank. This has clearly indicated that the spawning response is depending on individuals and their conditions rather than generalization. The present results offer some means of maximizing, maturation and spawning response of sea bass through hormone treatment. This advantageous technique of hormone pellet implantation which is easy and cheap to fabricate and implant is useful to maximizing the effect of prolonging and elevating hormone levels for successful maturation of captive land based broodstock of sea bass. The administration of optimum dose of LHRH-a hormone would be useful in the successful spawning.

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Table 1. Influence of LHRHa on induction of ovarian development in *Lates Calcarifer* (Bloch)

Fish No.	Date	Length (mm)	Weight (kg)	Initial mean ova diameter (μ)	Hormone	Dosages μg/kg	Response
01.	04.06.'97	860	10	391	LHRHa Pellet	100	During last week of June '97, after 20 days of LHRHa Pellet implantation, mean ova diameter measured was 473μ.
02.	04.06.'97	820	9	363	LHRHa Pellet	100	After 38 days, during first week of July '97, ova mean diameter increased to 458μ.
03.	04.06.'97	650	6	346	LHRHa Pellet	100	After 49 days, during third week of July '97, the oocyte mean diameter measured was 454μ.
04.	04.06.'97	790	8	354	No hormone Pellet implantation (Control)		After 45 days, (July '97), the mean ova diameter was increased to 398μ. However, the ova attained the mean size of 451μ during first week of September '97 only after 80 days.
05.	04.06.'97	760	7	355	No hormone Pellet implantation (Control)		Mean oocyte diameter was found to be 402μ during July '97 and increased to 464μ during first week of October '97 only after 109 days.

Table 2. Details of induced breeding experiments in *Lates Calcarifer* (Bloch)

Expt. No.	Date	Length (mm)	Weight (kg)	Mean ova diameter (μ)	LHRHa dose		No. of eggs spawned (million)		Spawning time after hormone injection (hr)	Rate of fertilization (%)		Rate of hatching (%)		Time taken for hatching after spawning (hrs)		Remarks
					Female	Male	First Spawning	Second Spawning		First Spawning	Second Spawning	First Spawning	Second Spawning	First Spawning	Second Spawning	
01	24 06 '97	860	.0	473	100	35	-				-			-		Plugged No spawning, even after 48 hrs of injection, stripping attempted But eggs not fertilized
02	12 07 '97	820	9	458	70	35	1 46	1 30	35	70		80		16		Spawning spontaneous and natural Second spawning observed 24 hrs after first spawning First spawned eggs successfully fertilized But, no fertilization in second spawned eggs
03	23 07 '97	650	6	454	60	35	1 05	1 00	34	80		90		17		Spawning spontaneous and natural First spawning eggs fertilized, second spawning observed 24 hrs after first spawning But, not fertilized
04	06 09 '97	790	8	451	70	30 35*	1 20	0 90	32	70	40	60	25	17	17	Spontaneous and natural spawning Eggs fertilized in both the spawning and hatching was successful For second spawning, fresh males were introduced with hormone treatment
05	05 10 '97	760	7	464	70	35 35*	1 10	1 00	34	80	60	70	53	17	17	Spawning spontaneous and natural Both first and second spawned eggs fertilized and hatched

* = In the second spawning LHRHa dose for fresh set of males