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INDUCED SPAWNING AND LARVAL GROWTH OF THE RABBIT FISH, *SIGANUS GUTTATUS* (OSTEICHTHYES; SIGANIDAE)

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Induced spawning of the rabbit fish, *Siganus guttatus* was accomplished with injections of reproductive hormones. Males and females were injected with testosterone and HCG; respectively. Eggs were hatched and larvae reared to the fry stage. Successful spawning was observed in experimental female fish by giving injections of human chorionic gonadotropin at a dosage rate of 500 Iu per treatment per fish (300g) at every 24 hrs until they spawned. Males received only one injection of testosterone at a dosage rate of 0.2cc/fish (300g). The rate of fertilization was > 90%. Hatching occured with in 22-27 hrs after fertilization at an ambient temperature of 26-30°, salinity of 32-36 ppt and dissolved oxygen of 5.2-6.1 ppm. Larvae were reared for 20 days by feeding them on *Isochrysis, Tetraselmis, Brachionus* sp. and *Artemia salina* nauplii. The overall survival rate of larvae was 0.71% and the mean length of survivors was 9.29 mm after 20 days.

Key words : *Siganus guttatus*, Induced spawning, Larval rearing.

Introduction

Rabbit fishes (family, Siganidae) are widely distributed in the Eastern Mediterranean, Indo-Pacific region [1] and in the Arabian sea [2]. of 30 known species, 15 are found in the Philippines Archipelago and 3 in Pakistan. *Siganus guttatus* is the most desirable of the three species viz. *S. guttatus, S. canaliculatus* and *S. argenteus,* which have a good potential for mariculture due to their high salinity tolerance (16-40 ppt), temperature fluctuations (26-30°), rough handling and crowded conditions [3-5].

As commercial scale production of this species requires consistent seed supply, breeding the fish in captivity would be the answer. Though information on induced spawning of few species of siganids is available [6-11], no report has been made on *S. guttatus* in this line. Hence, the present study was conducted at the South East Asian Fisheries Development Centre, Aquaculture Department of Philippines, on April 19, 1983 to induce the fish to spawn by hormone injections (viz. Human chorionic gonadotrop in and testosterone) and rear larvae to the fry stage.

Materials and Methods

Adult males and females of about 300 g each were collected from the broodstock tank of the finfish hatchery at SEAFDEC, Aquaculture Department of Philippines during the first quarter of the moon on April 19, 1983. Fish were selected for hormone treatment on the basis of external appearance. Female were distinctly plump and had an enlarged body, males were selected that had milt freely oozing from the urogenital pore when pressed lightly in the abdominal area. The stage of maturity of females was determined from the mean diameter of oocytes, collected by using polyethylene cannula [12]. Females having oocytes at the tertiary yolk globule stage were selected for the experiment. Males having white and creamy milt were considered mature. Males and females were then transferred into circular fiberglass tanks (600 1 capacity each) with the ratio of 3:1 respectively. Tanks were filled with filtered seawater having a salinity of 34 ppt and provided with aeration.

Four females were anaesthetized by submersing them into a 20-1 pail of seawater containing 2-Phenoxyethanol at a concentration of 100 ppm. Females were injected intramuscularly below the dorsal fin with 500 IU of HCG every 24 hrs until they spawned. Males were given an injection of 0.2CC testosterone/fish once on day that females were first injected. Sham-injected and untreated fish were kept in the tanks 2-4 days after all the treated fish with corresponding mean egg diameter had spawned. After injections, fish were transferred back to the spawning tanks. Water was changed daily to remove the nitrogenous wastes, mucus, faeces and some dirt until they spawned. After spawning, fish were removed from the spawning tank and the fertilized eggs were allowed to hatch. Embryonic developmental stages were studied under the microscope. The rates of egg fertilization, hatching and larval survival were determined *S. guttatus* produced > 120,000 eggs/fish.

Newly hatched larvae from one spawner were transferred to 8 rearing tanks of 600 1 capacity containing 400 1of aged and filtered seawater supplied with gentle aeration. Larvae were stocked at a density of 30/1 (12,000 larvae/tank) and reared up to 20 days. The experiment was conducted in a semi-enclosed hatchery and the tanks were further sheltered with translucent plastic sheets to prevent excessive sunlight and temperature fluctuations. The external walls of the tanks were wrapped with thin black plastic material to provide contrast and assist the larvae in locating prey. On day 1, no feed was given as the larvae would depend completely on the endogenous food. One day 2, *Isochrysis galbana, Tetraselmis*

chuii and *Brachionus plicatilis* were introduced at an initial density of 3-3.5x10^4 cells/ml and 20 cells/ml respectively. *Chlorella virginica* was introduced on day 12 at the concentration of 1-2 x 10^6 cells/ml. Starting on day 16, day old *Artemia salina* nauplii were added at a density of 2-3 animals/ml (Fig. 1) Temperature, dissolved oxygen and salinity were measured by using thermometer, Do-meter, YSI model 57 and a temperature compansated A-O refractometer in the morning and afternoon. One hundred litres of water per tank was changed daily with conditioned and filtered seawater.

Results and Discussion

Experimental fish spawned by injections of HCG and males by injections of testosterone. Results of the induced spawning of fish are presented in Table 1. Females received number of HCG doses according to the mean diameter of the oocytes. The sham-injected fish with mean egg diameter from 0.40 mm to 0.43 mm did not spawn. The mean diameter of the oocyte before and after spawning was recorded as<0.47 mm and 0.50 mm respectively. Fertilized eggs were approximately 0.60 mm in diameter, transparent, demersal, adhesive and with many oil globules while unfertilized ones were non-transparent, nonadhesive, buoyant and without oil globules. About 22-27 hrs were required to complete embryonic development from fertilized eggs to hatching at a temperature range of 26-30°, salinity range of 32-36 ppt and dissolved oxygen of 5.2-6.1 ppm. Table 2. describes the stages of embryonic development till hatching, observed under the microscope. In all the cases, when eggs were fertilized, fertilization and hatching rates were more than 90%. The mean total length of the newly hatched larvae was 2.28+ 0.96 mm.

The temperature in the rearing tanks, varied from 26.9-28.5° in the morning to 26-29°. 29,5° in the afternoon. Dissolved oxygen in the tanks varied from 5.2-6.0 ppm in the morning to 5.4-6.1 ppm in the afternoon and salinity ranged from 32-36 ppt.

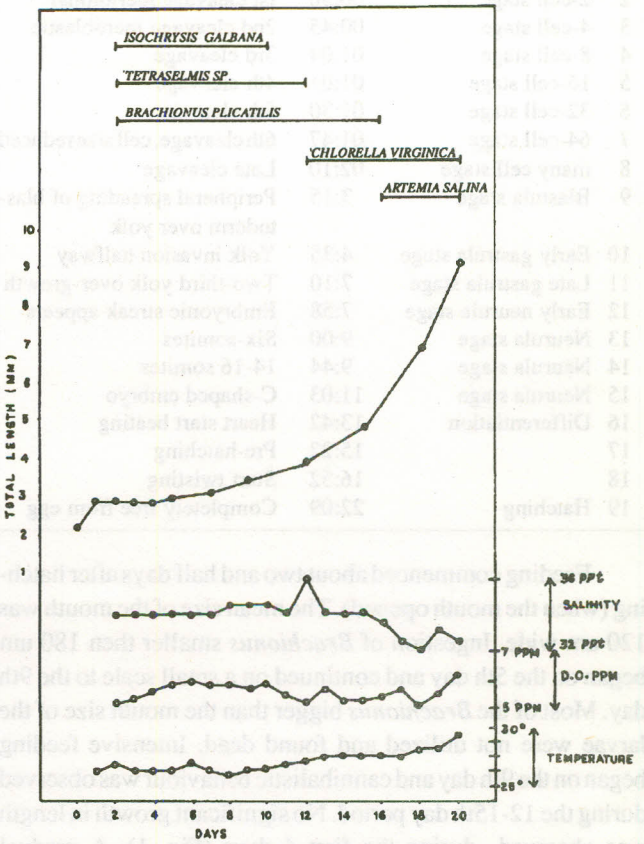

Fig. 1. Feeding scheme, water temperature, dissolved oxygen variation and growth of larvae for 20 days rearing period.

TABLE 1. DATA ON INDUCED SPAWNING OF *SIGANUS GUTTATUS* AT AMBIENT TEMPERATURE (26-30°) AND AT A SALINITY OF 32-36 PPT.

Fish	Initial egg diameter (mm)	Injection		Total doses at 500 IU HCG per injection	Remarks and observations
		No. of doses	Time intervals		
F1	0.39	5	24	2500	Fish spawned 11hrs after injection: Fertilization rate 98%, Hatching rate, 99.9%, Hatching period 22-25 hrs.
F2	0.43	3	24	1500	Fish spawning 14 hrs. after injection, Fertilization rate 98.06%, Hatching rate 99%, Hatching period 22-27 hrs.
F3	0.40	5	24	2500	Fish spawned 16 hrs. after injection; Fertilization rate 98%, Hatching rate 99%, Hatching period 22-27 hrs.
F4	0.41	4	24	2000	Fish spawned 17 hrs. after injection; Fertilization rate 99%; Hatching rate 99%, Hatching period 23-25 hrs.
F5	0.40	0	–	–	Untreated fish; did not spawn.
F6	0.43	0	–	–	Untreated fish, did not spawn.

TABLE 2. EMBRYONIC DEVELOPMENT OF *SIGANUS GUTTATUS*.

Plat No.	Stae of development	Time hrs: min.	Description
1	Fertilized egg	00.00	Spherical, transparent, adhesive, with oil globules
2	2-cell stage	00:30	Ist cleavage meridional
3	4-cell stage	00:45	2nd cleavage meroblastic
4	8-cell stage	01:04	3rd cleavage
5	16-cell stage	01:01	4th cleavage
6	32-cell stage	01:30	5th cleavage
7	64-cell stage	01:47	6th cleavage, cell size reduced
8	many cell stage	02:10	Late cleavage
9	Blastula stage	3:15	Peripheral spreading of blastoderm over yolk
10	Early gastrula stage	4:35	Yolk invasion halfway
11	Late gastrula stage	7:10	Two-third yolk over-growth
12	Early neurula stage	7:58	Embryonic streak appears
13	Neurula stage	9:00	Six-somites
14	Neurula stage	9:44	14-16 somites
15	Neurula stage	11:03	C-shaped embryo
16	Differentiation	13:42	Heart start beating
17		15:22	Pre-hatching
18		16:52	Start twisting
19	Hatching	22:09	Completely free from egg

Feeding commenced about two and half days after hatching (when the mouth opened). The mean size of the mouth was 120 um wide. Ingestion of *Brachionus* smaller then 180 um began on the 5th day and continued on a small scale to the 9th day. Most of the *Brachionus* bigger than the mouth size of the larvae were not utilized and found dead. Intensive feeding began on the 9th day and cannibalistic behaviour was observed during the 12-15th day period. No significant growth in length was observed during the first 4 days (Fig. 1). A gradual increase in growth was observed from 5th to 9th day. This growth period coincided with the onset of larval feeding (5-8th day) and intensification of feeding on the 9-12 day respectively. Rapid growth in length was observed as soon as the larvae opened their mouths. This could be due to the absence of the food organisms of the required size. Larvae owing to their small mouth size, can consume only the smaller rotifers measuring less than 125 um. This poses a problem as only a smaller proportion of the rotifer population fed to the larvae constitutes this size group, which is virtually only the newly hatched rotifers. The poor food intake by the larvae especially during their early stages of development could also be attributed to the inability of level to see the food properly as the high density of phytoplankton was hardly maintained at the required level in this experiment. The nutritive value of food taken by the level is also one of the detemrining factors in larval survival [13]. The remaining larvae after day 12 showed a significant increase of growth when fed with one day old *Artemia* nauplii but the only drawback is its high cost. Survival rate of the larvae was apparent as the larvae passed the 9th day. Two critical periods of high mortality were the days 3-4 and days 10-13. These two periods accounted for 60% and 30% mortalities. The overall survival rate on 20th day after hatching was 0.71% and survivors measured 9.29 mm in length.

Results obtained in this study indicate the possibility of artificial propagation of captive *S. guttatus* by hormone injection (HCG). Lam, [1] has also successfully used HCG to induce spawning in *S. canaliculatus*. The response of captive adult female *S. guttatus* to HCG depends largely on the initial development stage of oocytes. Shehadeh, *et al.* [14] also reported for the grey mullet, that the amount of HCG required to induce spawning varies inversely with the mean egg diameter of the recipient female. Natural spawning, following HCG treatment has been observed in this experiment May, *et al.* [10]. Bryan, *et al.* [11] and have also reported natural spawning in case of *S. canaliculatus*.

The most important factor in rearing larvae of marine fishes is considered to be the provision of adequate and suitable food [1, 10, 15]. Two critical periods involving high mass mortality of larvae co-incided with the opening of the mouth or when the yolk and oil globules were completely absorbed (Day 3-Day4) and second growth phase (Day 10-13). Lack of feeding was 0.71% at the end of this experiments. Similar results of larval growth and lower survival rate were obtained for *S. canaliculatus* [10,16,17].

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