Observations on the Spawning, Larval Development, and Larval Rearing of Siganus argenteus (Quoy and Gaimard) under Laboratory Conditions*

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Fishes of the family Siganidae have been identified as potential candidates for culture because of their desirability as human food and their herbivorous feeding habits (Bryan and Madraisau, 1977). However, a major problem is the unavailability of a substantial number of fry for stocking purposes (Popper et al., 1976). Soh and Lam (1973) stated that successful fish culture would depend on a regular supply of fry for stocking. In the Philippines, siganid fry for culture are obtained from nature, which may not be a reliable source on a long-term basis, as undesirable upsets in the population dynamics of the fish may be caused (Soh and Lam, 1973). Popper et al. (1976) suggested a solution to this problem: breeding the fish artificially.

Several studies have reported on the artificial breeding of siganids. Manacop (1937) studied the artificial fertilization of *danggit, Amphacanthus oramin* (= *Siganus canaliculatus*), using the wet and dry methods. Soh and Lam (1973) succeeded in the artificial fertilization of eggs from sexually mature *Siganus oramin* (= *Siganus canaliculatus*) injected with human chorionic gonadotropin (HCG). Westernhagen and Rosenthal (1975) did experiments on the artificial fertilization of eggs from hormone-induced *S. canaliculatus* in five different salinities. Popper et al. (1976) succeeded in spawning and hatching *S. vermiculatus* using HCG injections.

This study aimed to determine the spawning seasons of *S. argenteus* under laboratory conditions without the use of hormones and to observe the development of the eggs and larvae under specific conditions of temperature and salinity. As yet, very little has been published on the spawning season, larval development, and larval rearing of *S. argenteus*, aside from a study on induced spawning by Burgan and Zselecsky (1979). Moreover, our knowledge of the development of siganid eggs and larvae has been confined to those of *S. canaliculatus* (Manacop, 1937; Soh and Lam, 1973; May et al., 1974; Westernhagen and Rosenthal, 1975), *S. vermiculatus* (Popper et al., 1976), and *S. lineatus* (Bryan and Madraisau, 1977). Up to this date, little success has been achieved in rearing larval siganids through metamorphosis. May et al. (1974) obtained a larval survival rate of only 0.3% with *S. canaliculatus*. Popper et al. (1976) obtained a 9% survival rate for *S. vermiculatus;* Bryan and Madraisau (1977) got 16% survival with *S. lineatus*.

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S. argenteus was chosen as the object in our study for a number of reasons, including their adaptability to various environmental factors, hardiness in the laboratory, relatively fast growth, and suitably for culture in floating and bottom sea cages (Burgan and Zselecsky, 1979).

Materials and Methods

Four adult *S. argenteus*, one male and three females, were used as brood stock in fourteen successful spawning occurrences under laboratory conditions within the period from August 1979 to July 1980. The male individual, which measured 161 mm standard length, was smaller and more slender that the female individuals, which measured 178 mm, 190 mm, and 192 mm standard length. During the spawning season, the females developed enlarged abdomens, which when slightly pressed exuded orange-colored eggs. These fish were collected as fry from Solong-on, Siquijor Island, using a beach seine. At collection they averaged about 26 mm standard length. They were reared in floating sea cages together with other siganid species for eight months, fed with filamentous green algae, corn bran, and dried leaves of *Leucaena leucocephala*. When brought to the Silliman University Marine Laboratory in January 1979, the fish were about ten months old, measuring an average standard length of 170 mm (Burgan and Zselecsky, 1979) Prior to our experiments they had already been observed to spawn three times in the laboratory.

The fish were held in an outdoor, rectangular concrete tank with a capacity of 4000 liters. The tank was provided with aerators, and running sea water flowed through at least eight hours a day. A blue translucent plastic roof covered the tank, to limit phytoplankton bloom, prevent water dilution by rain, and provide shade on hot afternoons. The average temperature of the water in the tank ranged from 26.4°C in the morning to 30°C in the afternoon, and the salinity ranged from 32 ppt to 34 ppt. The fish were fed daily with green algae, *Enteromorpha* spp. and *Rhizoclonium* spp.

On the day before the spawning time — two to four days following the new moon — the spawning tank was cleaned and then filled with filtered, fresh sea water. The fish were not given food, and at approximately 4 p.m. the water was lowered to about 10 to 15 cm and left overnight. On the following morning the water was examined for eggs.

After the fish spawned, they were removed and temporarily transferred to a glass aquarium. The tank was allowed to refill with filtered fresh sea water and provided with vigorous aeration until the eggs hatched.

Temperature and salinity were monitored twice daily using a mercur thermometer. Three samples of eggs were measured with a micromete eyepiece and their development through hatching observed under the micr scope.

The hatched larvae (approximately 12 hours old) were stocked i two rectangular, concrete (4000 liter capacity) rearing tanks and thr 200-liter glass aquaria. The number of larvae stocked in each tank w 16,000, about four larvae per liter of water. Five hundred larvae, abo three per liter of water, were stocked in each aquarium.

Prior to stocking, the rearing tanks and aquaria were filled wit filtered, fresh sea water and phytoplankton and small rotifers were added The phytoplankton was cultured in a 3000-liter concrete tank, while th rotifers (*Brachionus plicatilis*) were cultured in small rectangular plast trays. The phytoplankton was a mixture of species dominated by u identified green flagellates (3.3 to 6.6 micra in diameter).

All rearing tanks and aquaria were provided with gentle aeration Water temperature was taken three times daily and salinity once a day Good water quality was maintained by supplying the tank with filtere sea water, flowing through at a rate of two liters per minute; in the aquari half of the water was removed and replaced with filtered fresh sea wat twice daily. The density of food was checked daily to determine feedin time of the larvae.

The growth and development of the larvae were monitored by takin three daily samples for examination under the microscope The differe developmental stages were photographed under low-power magnificatio

Results and Discussion

Spawning.

Our fish spawned fourteen times in the tank (Table 1). The spawning occurred once at the new moon, six times 1 or 2 days before and aft the new moon, three times 3 or 4 days before and after the new moon, a four times 5 to 7 days before and after the new moon. These spawning occurred even without the level of the water in the tank being lowered However, in August 1979 and in February and May 1980 the fish spawne immediately after being replaced in the tank from which they were remove while it was cleaned. On the other hand, spawnings in March, April, a June 1980 were unexpected. The tank had not been cleaned and the wat not changed; food and fecal material were mixed with the buoyant eggs i

the water. These spawnings were discovered because of the very strong fishy odor in the tank after the fish spawned. The same observation was made immediately after each spawning. For all spawnings the temperature of the water in the tank varied between 27 and 28°C and the salinty was 34 ppt.

Table 1. Spawning season of Siganus argenteus under laboratory conditions within the period from August 1979 to July 1980

Period of observation	Spawning time	new moon	Number of days before new moon	Number of days after new moon
August 1979	August 24	August 22		2
February 1980	February 18	February 16		2
March 1980	1) March 14	March 16	2	
(3 times)	2) March 15		1	
	3) March 16			
April 1980	1) April 10	April 5	5	
(3 times)	2) April 11		4	
	3) April 12		3	
May 1980	1) May 16	May 14		2
(3 times)	2) May 17			3
	3) May 21			7
June 1980	1) June 17	June 12		5
(2 times)	2) June 19			7
July 1980	July 11	July 12	1	

Note: It is probable that spawnings occurred during the months of September to January but were not discovered.

Our experiments proved that twelve-month-old *S. argenteus* with an average standard length of 17 cm are sexually mature and, like *S. vermiculatus* (Popper et al., 1976), capable of spawning in captivity without the use of hormones. Apparently though, the repeated lowering of the water level in the tank for a few hours to a few days induced *S. argenteus* to spawn during three occurrences. This procedure apparently simulated the rise and fall of the tide levels which presumably influence

the spawning of siganids in the natural environment. Manacop (1937) observed that *Siganus canaliculatus* come in large schools to shallow tidal flats as the tide begins to rise and spawn after midnight, as the tide begins to recede. In February 1980, the siganids might have been induced to spawn by stress resulting from their being handled when the tank was cleaned.

This study further showed that *S. argenteus* spawns around the new moon, from one week before to one week after. The spawnings that occurred within the week after the new moon conform with the spawning season of *S. canaliculatus*. Manacop (1937) reported that at Bantayan Island, Cebu, *S. canaliculatus* spawned from the fourth to the seventh night after the new moon and in Murcielagos Bay and near Tagbilaran, Bohol the spawning period usually lasted for two to three days, beginning on the fifth day after the new moon. Consequently, the spawning of *S. argenteus* comes ahead of that of *S. vermiculatus,* which probably occurs on or one night after the first quarter phase of the moon (Popper et al., 1976), and that of *S. lineatus,* which occurs three days preceding the full moon (Bryan and Madraisau, 1977).

The temperatures and salinities at which the fish spawned fall within the ranges of those observed in other studies. *S. argenteus* spawned at 27 to 28°C and 34 ppt salinity. Soh and Lam (1973) observed that *S. canaliculatus* spawned at 25 to 27°C and 26 to 28.5 ppt salinity, *S. vermiculatus* spawned at 26 to 30°C and 29 to 33 ppt salinity (Popper et al., 1976), and *S. lineatus* at 25.5 to 31.6°C and 25 to 31.5 ppt salinity (Bryan and Madraisau, 1977).

Incubation Period and Hatching.

The eggs of *S. argenteus* were transparent, non-adhesive, and buoyant or free-floating in the water column. The egg diameter ranged from 294 to 700 micra (0.29-0.70 mm.); the egg contained a large oil globcle. A few hours prior to hatching, the embryo within the egg membrane exhibited several twisting movements. It was slightly elliptical in shape; the oil globule was centrally located and distinctly bounded by the yolk; and the primordial head, dorsal fins, somites, and tail parts were visible. Later, the optic vesicle and the tail bud developed and the heart began to beat rapidly. Then the embryo rotated intermittently within the egg membrane as a result of which it became curled up, with the head and tail meeting each other. During this time, the embryo continued its twisting movements which enabled it to break out of the membrane and finally emerge as

newly-hatched larva. The larvae began hatching late in the evening; hatching probably lasted for about one hour.

The eggs of *S. argenteus* were unlike those of *S. canaliculatus* (Soh and Lam, 1973; Manacop, 1937) and *S. vermiculatus* (Popper et al., 1976), which were demersal and adhesive and contained several oil globules. The diameter of the eggs of *S. argenteus* measured from 0.29 to 0.70 mm, comparable to those of *S. canaliculatus* (Manacop, 1937; Soh and Lam, 1973; May et al., 1974) and *S. vermiculatus* (Popper et al., 1976).

Our experience with *S. argenteus* confirms the findings of Burgan and Zselecsky (1979) that the incubation period of *S. argenteus* eggs is 24 to 26 hours, like that of *S. canaliculatus* (May et al., 1974), *S. lineatus* (Bryan and Madraisau, 1977), and *S. vermiculatus* (Popper et al., 1976). In another study, Westernhagen and Rosenthal (1975) reported that the incubation time of *S. canaliculatus* in various salinities was between 27 and 29 hours, while Soh and Lam (1973) observed it to last from 30 to 35 hours.

At 26.4 to 28°C and 32 to 34 ppt salinity, the hatching success of the eggs of *S. argenteus* per spawning time in the tank was approximately 80%. Soh and Lam (1973) obtained about the same high percentage of successful hatching with *S. canaliculatus*. Westernhagen and Rosenthal (1975) noted a hatching success of *S. canaliculatus* that equalled or surpassed 95% at 29.9 to 32.2 ppt salinity, but was only 3.6% in 15.8 ppt salinity.

Larval Development and Behavior.

The newly-hatched larvae measured from 1.54 mm to 1.68 mm in total length. The yoke sac was about half the body length, with the oil globule at its center. The body was transparent and slightly bent, twisting occasionally with tail and head meeting (Fig. 1a and b). It assumed its bent position until 18 hours old. On Day 1 (the first day after hatching) the average total length of the larvae was 2.18 mm (Fig. 2). Melanophores were present on the snout, anterior portion of the yolk sac, and posterior portion of the anus. The eyes had very little pigmentation; the gut was a simple straight tube. The larvae were actively swimming to the surface and then passively floating down, occasionally jerking either toward the surface or downward in the water column. They were positively phototactic; that is, they tended to swarm readily towards a source of light.

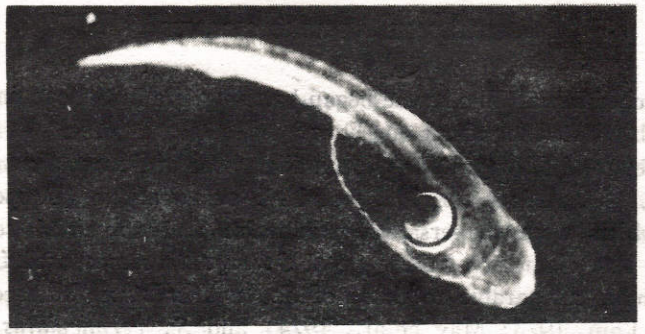

Figure 1. a. (above) Newly-hatched (ten minutes old) *S. argenteus larva.*

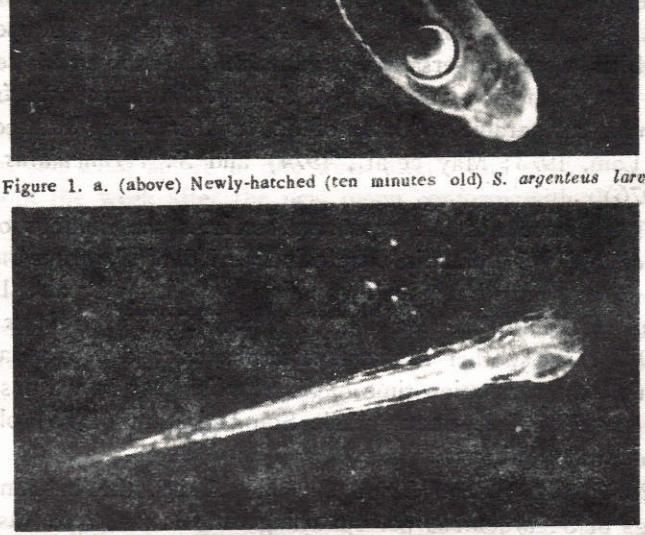

Figure 1. b. (below) Eighteen hour old *S. argenteus* larva.

Figure 2. One day old *S. argenteus* larva.

Figure 3. Three day old *S. argenteus* larva.

On Day 2, the larvae measured 2.21 mm average total length. The eyes were fully developed and heavily pigmented; the yolk sac was completely absorbed and the oil globule much reduced; the gut was looped and complete; pectoral fins were formed; rudimentary gills were visible and the jaws were fully formed and functional. The larvae exhibited a snatching-feeding behavior which was observed frequently, at intervals of only a few seconds, near the sides and bottom of the aquaria. They appeared to be feeding on phytoplankton; their guts contained some green materials, presumably green flagellates.

By Day 3, the larvae had grown to an average total length of 2.38 mm. The pectoral fins were fully developed and functional; gill covers were formed; and the caudal fins had started development (Fig. 3). They had begun feeding on rotifers; these were found in their guts.

In general, the development and behavior of *S. argenteus* larvae observed within a few days after hatching were similar to those of *S. canaliculatus* (Manacop, 1937; Westernhagen and Rosenthal, 1975; Soh and Lam, 1973). However, the total length of the newly-hatched *S. argenteus* larvae was much less than that of *S. lineatus*, 2.5 mm standard length (Bryan and Madraisau, 1977). Westernhagen and Rosenthal (1975) showed the total length of *S. canaliculatus* at hatching to be around 2 mm. May et al. (1974) measured 2.1 mm and Manacop (1937) measured 1.5 mm total length of the same species. Newly-hatched larvae of *S. vermiculatus* were about 1.8 mm long (Popper et al., 1976).

Our data also indicated that three days after hatching the average total length of *S. argenteus* larvae was 2.38 mm. Soh and Lam (1973) reported that the larvae of *S. canaliculatus* attained a total length of 2.33 mm about two days after hatching. This suggests that under laboratory conditions *S. argenteus* larvae grow slower than *S. canaliculatus*.

It was evident from the various food counts that on the second day after hatching, the larvae of *S. argenteus* preferred phytoplankton to rotifers (*Brachionus plicatilis*) and on the third day they consumed both phytoplankton and rotifers. Rotifers are among the food organisms recommended for feeding larval marine fishes in the laboratory (May 1970). Obviously, the size of food organisms that can be ingested and digested by the larvae is related to their developmental stage. In another study, Byran and Madraisau (1977) gave rotifers to the larvae of *S. lineatus* from Day 2 until Day 14. From then on they were given copepod nauplii and *Artemia* nauplii. May et al. (1974) observed that the larvae of *S. canaliculatus* began feeding on rotifers on Day 4.

Larval Mortality.

In the tank with water at 34 ppt salinity and 26 to 30°C temperature, the larvae of *S. argenteus* died the third day from hatching while those in the aquaria died one day earlier.

The present study confirms the generalization that the main obstacle to the artificial propagation of many marine fish is mortality during larval stages (Popper et al., 1976). In two to three days, the mortality rates of *S. argenteus* larvae were 100% both in tanks and aquaria. However, causes of death were not clearly determined. In the aquaria, the larvae probably died due to water fouled by the accumulation of dead food organisms which promoted the growth of bacteria. Examination of water samples revealed that there were some dead rotifers present. On the other hand, the larvae in the tanks might have died due to overcrowding, fluctuating temperatures, or starvation, in spite of the high density of food given. The larvae might have ingested particles of food too large for them to digest.

Our experiments support the findings of Bryan and Madraisau (1977) that most deaths occurred between Days 1 and 4. Although the dead larvae had food in their guts, it might not have been a type suitable for growth and survival.

Conclusions and Recommendations

Sexually mature *Siganus argenteus* are capable of natural spawning in captivity — that is, without the injection of hormones. In contrast to induced spawning, natural spawning would be an inexpensive method of producing siganid fry for stocking in floating sea cages, fishpens, and ponds. However, reduction of the water level in the spawning tank repeatedly prior to the spawning season or subjecting the gravid siganids to some form of stress apparently also induces them to spawn.

The results of our study indicate that *S. argenteus* does not spawn throughout the year, unlike *S. canaliculatus* and *S. spinus* (Laviña and Alcala, 1974). Under laboratory conditions, *S. argenteus* spawns from February until August. However, it is probable that spawning also occurs in the months of September to January, before the new moon. Our spawners probably spawned during these months but were not discovered because our observations were made only after the new moon, while spawning may occur up to one week before or after the new moon.

In order to obtain a high percentage of success in hatching, the spawn-

ing tank should be cleaned and, preferably, the siganids should not be given food for about two days before the expected spawning time. This will prevent clumping of the eggs with food and fecal materials, creating considerable problems in incubation.

The incubation period of *S. argenteus* lasts for 24 to 26 hours, as in *S. canaliculatus* (May et al., 1974), *S. vermiculatus* (Popper et al., 1976), and *S. lineatus* (Bryan and Madraisau, 1977). The behavior and development of the larvae a few days after hatching are similar to those of *S. canaliculatus*. It was found that at 26 to 30°C and 32 to 34 ppt salinity, *S. argenteus* larvae grew slower than *S. canaliculatus* (Soh and Lam, 1973).

Our observation showed that an abundant supply of suitable food is essential for successful larval rearing, especially in large rearing tanks. Since young larvae can ingest only small prey, it is necessary that an adjustment of the food regime be made according to the developmental stage of the larvae. Ciliates and rotifers or phytoplankton may be given from the second day until about a week after hatching. Copepods and other bigger food organisms may be given later in the larval period. Wild plankton is a good supplement to larval food since it is easy to collect and it contains the food which fish larvae feed on under natural conditions; but plankton may also be disadvantageous because of the difficulty of removing organisms which may prove harmful to the larvae (May, 1970).

Probable causes of the mortality of the larvae observed in both tanks and aquaria include (1) fouling of the water due to the accumulation of uneaten food organisms, which promoted the growth of bacteria, (2) unsuitable food for the larvae, and (3) temperature and salinity which may not have been optimum for larval growth and survival.

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