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NATURAL SPAWNING AND REARING OF MANGROVE RED SNAPPER, *LUTJANUS ARGENTIMACULATUS*, LARVAE IN CAPTIVITY

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Abstract

Mangrove red snapper *(Lutjanus argentimaculatus,* Forsskål) spawned naturally in captivity, without the use of hormones or other treatments, from May 21 to September 15, 1999. Each female laid an average 2,350,000 eggs. Larvae were reared in 4-ton circular fiberglass tanks. They were first fed S-type rotifers (*Brachionus rotundiformis*). Later, *Artemia* nauplii and copepods were added to the diet. They were weaned onto an artificial diet. Metamorphosis began at 18 days when the larvae reached 10.5 mm total length and was complete by day 30 when larvae were 17.2 mm. From day 26, large larvae (over 25 mm) cannibalized their smaller siblings. Abnormalities were observed in 4.9% of the individuals. At 50 days, the larvae rearing trial produced juveniles of 49 mm average total length with a survival of 10.8-32.3%.

Introduction

The snapper family Lutjanidae, a gonochorist (Grimes, 1987), contains many commercially important species of tropical and subtropical coastal fisheries. The mangrove red snapper *Lutjanus argentimaculatus* (Forsskål) is widely distributed in the Indo-West Pacific from Samoa and the Line Islands to East Africa, and from Australia northward to the Ryukyu Islands (Allen, 1985). It is a marine species but is also found in brackish mangrove estuaries and the lower reaches of freshwater streams. It migrates offshore to deeper reef areas, sometimes penetrating depths exceeding 100 m (Allen, 1985, 1987). It is a good

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candidate for mariculture because of its high economic value and ability to adapt to various salinities and temperatures (Chen et al., 1990; Estudillo et al., 2000). The spawning season varies among localities. In Thailand, spawning occurs between January and November, at a temperature of 27.0-32.8ºC (Singhagraiwan and Doi, 1993). In Taiwan, spawning occurs between April and October (L.-T. Lin, pers. comm.), and in the Philippines in August (Emata et al., 1994). This species is economically important to Asian coastal aquaculture and fisheries. It is a high-priced food fish (about US$7/kg wholesale) and the demand exceeds the supply.

During the past decade, much of the interest in mariculture of *L. argentimaculatus* focused on problems related to spawning and larvae rearing. The first technical breakthrough in induced spawning and larvae rearing of *L. argentimaculatus* was achieved by Wudthisin (1984) in Thailand. The complete morphological development of eggs, larvae and juveniles has been described by Doi et al. (1994). However, natural spawning of *L. argentimaculatus* in captivity has not yet been reported. As with many other mariculture species, there are still many problems and issues waiting to be solved in the larvae rearing of *L. argentimaculatus*. Presently, this species is being cultured on a commercial scale in Taiwan and other Asian countries (Chou et al., 1995; Liao et al., 1995), but no information has been made public by these companies about the techniques they use to procure red snapper eggs and produce fry. The present study reports the successful results of natural spawning of *L. argentimaculatus* during the 1999 breeding season, and describes a pilot-scale larviculture system.

Materials and Methods

Broodstock maintenance. Juvenile *L. argentimaculatus,* collected from the wild, were reared for seven years to produce broodstock. Thirty broodfish (14 females and 16 males) were maintained in a concrete pond (3000-ton capacity, 3 m water depth) with a salinity of 30-33 ppt and temperature of 22.8-25.5ºC where they acclimated to captive conditions for about three months prior to spawning. The females weighed 6.92-8.36 kg and ranged 72.4-77.0 cm fork length. The males weighed 6.48-7.42 kg and ranged 67.1-73.4 cm fork length. Mature females were easily recognizable by their swollen belly, whereas mature males were recognized as those from which milt emerged after light pressure on the belly. Water was continuously exchanged at a rate of about 40% per day. Lighting was ambient. The broodfish were fed trash fishes, such as carangid and scombrid spp., and squid (*Illex argentinus*). In general, the fish were fed at a minimum of 3% of their body weight per day.

Spawning and egg production. Fertilized eggs were collected from the spawning pond with a fin dip net (100 µm mesh). The eggs were transferred to six 4-ton circular fiberglass tanks. The eggs were incubated in mesh baskets (350 µm mesh) equipped with independent airlift systems to keep the eggs in suspension. The number of eggs was estimated volumetrically. Fertilization and hatching rates were estimated with 100 eggs from each spawn in a 1000-ml beaker of sea water. The fertilization rate was determined as the percentage of normally developing eggs 10 h after fertilization. The hatching rate was determined as the percentage of fertilized eggs which hatched.

Larvae rearing. Red snapper larvae were stocked at a density of 5-16.4 per liter. The feeding schedule is shown in Fig. 1. Rotifers (*Brachionus rotundiformis*) were prepared in cultures fed baker's yeast (*Saccharomyces cerevisiae*). Three to five days before being fed to the larvae, the rotifers were intensively fed microalgae (*Nannochloropsis* sp.). Together with the rotifers (110-210 µm lorica length), 6 l of *Nannochloropsis* were added to the larvae tanks as feed for the rotifers. Later, newly-hatched *Artemia salina,* copepods collected from the wild and a microcoated artificial diet (400-700 µm particle size; 40% protein; 27.8% lipid; 1.8% n-3 highly unsaturated fatty acids; Leu and Liou, 1992) were added to the diet.

Environmental conditions. Salinity during incubation and rearing was 30±2 ppt, while water temperature ranged 24-30ºC. Water

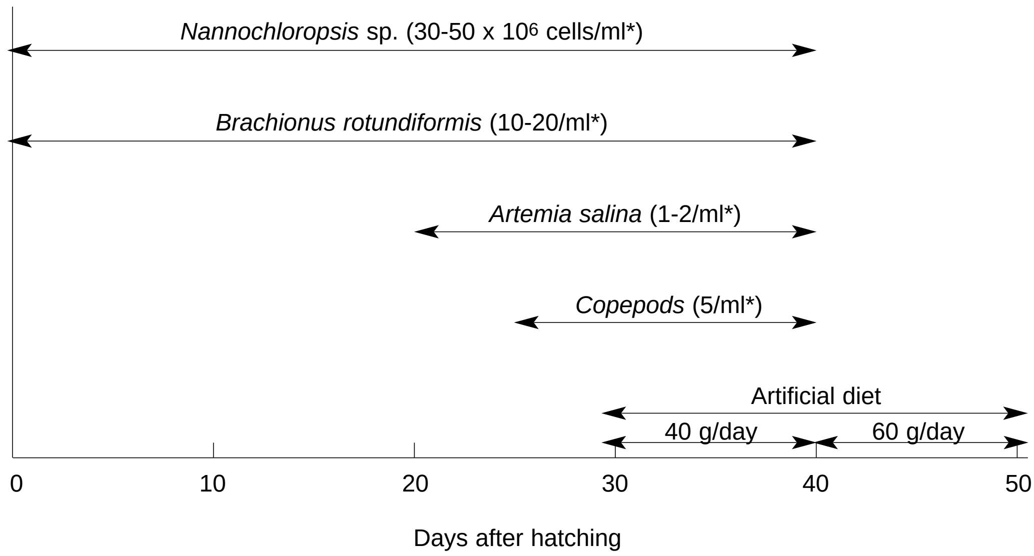

Fig. 1. Feeding scheme for larval rearing of the mangrove red snapper *Lutjanus argentimaculatus*.
* Density at which feed was maintained.

was maintained as a static system with very mild aeration (5-10 ml/min) until 10 days after hatching. After day 10, sea water was replaced at 10% per day. From day 21 to 40, when the fish were fed *Artemia* nauplii, one third of the rearing water was changed once daily. When the larvae started feeding on the artificial diet, running water at a rate of about 15-20 l/min was applied to prevent water quality problems. Debris, unhatched eggs and dead larvae were siphoned from the tanks daily. Dissolved oxygen, salinity, temperature, pH and live foods counts were also monitored daily. At the end of each rearing trial, the surviving juveniles were counted. The correlation between survival and the initial larvae stocking density was computed. After 50 days, all surviving juveniles were transferred to an outdoor earthen pond (2200 ton) for further rearing.

Results

Natural spawning. The broodstock spawned naturally five months after stocking, from May 21 until September 15, 1999. Spawning usually occurred between 23:00 and midnight. Daily spawning (Fig.2) for the 71 days on which spawning occured ranged 1057-186,571 eggs per female (average 33,571), equal to 2350 x 10^3 eggs per female over the period. The total number of eggs collected was 32,900 x 10^3, of which 24,438 x 10^3 were fertilized (74.3%). The hatching rate varied 34.8-99.5% with an average of 81.9%. Throughout the period, water temperature fluctuated 26.0-31.5ºC.

Fertilized eggs of *L. argentimaculatus* are transparent, spherical and pelagic, measure 0.74-0.81 mm in diameter, have a narrow perivitelline space, a clear, unsculptured chorion, a homogeneous and unsegmented yolk, and a single oil globule (0.14-0.16 mm diameter) at the vegetal pole. The eggs hatched at 25.8-28.7ºC, 16-22 hours after fertilization.

Larvae rearing. Newly hatched larvae measured 1.62-1.94 mm in total length (avg 1.78 mm) and had 24 pairs of myotomes. The larvae had large yolk sacs that extended forward from the snout. The yolk sac was fully resorbed 66-90 h after hatching, at which time the mouth began to open and the eyes were

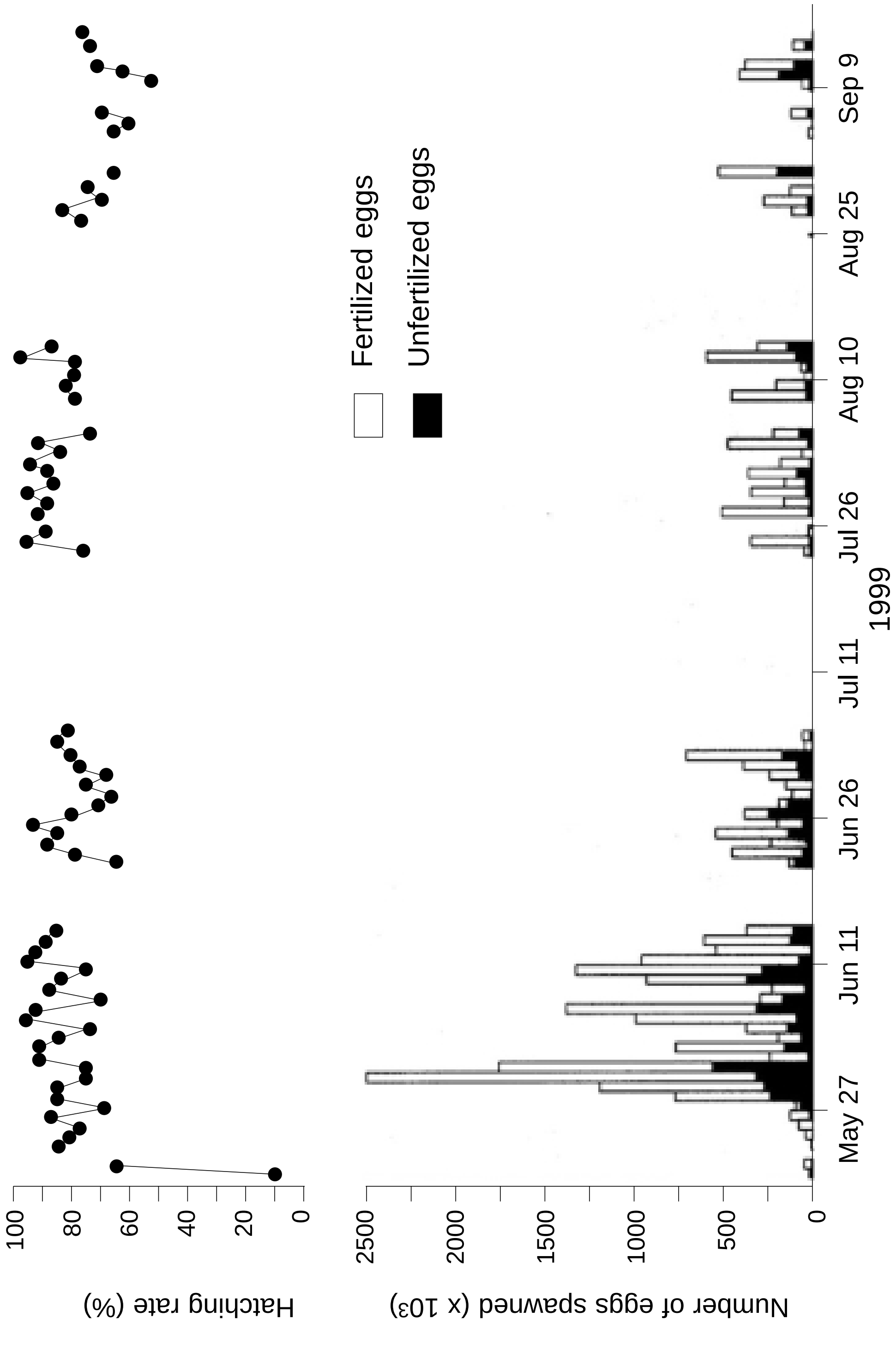

Fig. 2. Daily number of eggs spawned by *Lutjanus argentimaculatus* during the 1999 spawning season. No. of spawners = 16 males and 14 females; water temperature = 26.0-31.5°C.

pigmented. Elongated spines of the second dorsal and pelvic fin, unique morphological characteristics of lutjanids, were observed in *L. argentimaculatus* from 5 to 30 mm. Elongation and spination of the fins may play a role in maintaining buoyancy and avoiding predators (Moser, 1981).

Metamorphosis of the larvae to the juvenile stage occurred at 10.5-17.2 mm. From about 26 days after hatching, a few of the larger larvae (over 25 mm) began to exhibit cannibalistic behavior. They chased and sometimes apparently bit each other's eyes and fins. Table 1 summarizes the results of the rearing trials at 50 days; the juveniles (n = 48,892) reached a mean total length of 49.4±4.3 mm with an average survival of 21.1%. Initial stocking densities and final survival rates were neither significantly nor inversely related ($p>0.05$, r = 0.17). Abnormalities were observed in a small percentage of individuals, including lordosis (3.8%) and brachyospondyliosis (1.1%).

Discussion

Hormone treatments are reliable methods of inducing spawning in *L. argentimaculatus* (Wudthisin,1984; Lim and Chao, 1993; Singhagraiwan and Doi, 1993; Emata et al., 1994, 1999). However, the present study demonstrates that *L. argentimaculatus* is one of the few marine fishes that will spawn voluntarily in captivity. Voluntary spawning has the advantage of not requiring handling of the broodstock, thereby minimizing stress to the brooders and reducing labor costs. Based on the monthly differences in number of spawning days and number of eggs spawned, it is possible that the gonads of *L. argentimaculatus* partially matured in May and fully matured from June to September. Singhagraiwan and Doi (1993) assumed that seasonal fluctuations of environmental criteria affect the maturation and spawning activity of *L. argentimaculatus*. Doi and Singhagraiwan (1993) also reported that broodfish release eggs at night, usually between 01:00 and 04:00, and that a single spawning event could last up to six days.

At present, with mass-cultured rotifers as the initial food, 100,000 black porgy (*Acanthopagrus schlegeli*) fry (13-14 mm) can be produced in a 45-m^3 tank with a sur-

Table 1. Summary of larvae rearing trials of mangrove red snapper, at 50 days.

Trial number	*Number stocked (per 4 m^3 tank)*	*Stocking density (no./l)*	*Survival (%)*	*Harvest density (no./l)*	*Total length (mm)*	*Abnormality (%)*
Trial 1	20,800	5.2	14.6	0.8	44.6	6.3
Trial 2	41,200	10.3	20.8	2.1	45.7	3.4
Trial 3	29,200	7.3	23.7	1.7	55.4	5.1
Trial 4	47,600	11.9	32.3	3.8	50.9	6.8
Trial 5	65,600	16.4	10.8	1.8	47.1	3.5
Trial 6	32,800	8.2	24.1	2.0	52.8	4.0
Mean	39,533	9.9	21.1	2.0	49.4	4.9
SEM*	15,814	4.0	6.9	1.0	4.3	1.5

* Standard error of the mean

vival rate of 18-75% (Leu, 1997). This intensive larviculture method aims at producing large numbers of larvae by intensively controlling the rearing environment. Duray and co-workers (1996) successfully reared *L. argentimaculatus* larvae on small L-type rotifers, *Brachionus plicatilis* screened through a 90 µm mesh plankton net. Survival during the first 14 days was 5.64%. However, it seems that unscreened L-type rotifers (180-300 µm lorica length) cannot be used for the initial feeding of *L. argentimaculatus* larvae because they are too large for the mouth opening. Mouth size is an important factor in larvae rearing; it can limit prey size (Shirota, 1970). The mouth opening of *L. argentimaculatus* larvae when they start feeding is about 210 µm (angle between the upper and lower jaws is 90º). Assuming that edible prey must be 50-75% of the mouth size (Shirota, 1970), prey must be no larger than 105-160 µm to be eaten by *L. argentimaculatus* larvae.

In this study, S-type rotifers were first observed in the larvae four days after hatching. The size of the rotifers made them suitable as food in the early larval stages of this fish (days 3-5). It would be desirable to develop a reliable technique for S-type rotifers in the larviculture system. Oyster eggs and trochophores are often provided as first feed for *L. argentimaculatus* in Taiwan, together with S-type rotifers (L.-T. Lin, pers. comm.). The survival rates are fairly consistent, although it is likely that oyster eggs and trochophores are nutritionally inadequate for larvae growth and survival (Doi and Singhagraiwan, 1993). Nutrition of *L. argentimaculatus* remains poorly understood. This has hindered the development of feed for *L. argentimaculatus*. Wu and Tang (1989) reported that juvenile *L. argentimaculatus* require 38-40% high quality protein (of which some must be animal protein). Catacutan et al.(2001) found that a diet containing 44% protein with a P/E ratio of 23.3 mg/kJ was optimum for *L. argentimaculatus* growth. Other aspects of the nutritional requirements of *L. argentimaculatus* need further investigation.

The survival rate of larvae from hatching to metamorphosis varied from less than 11% to 32%. High mortality during larval stages is the main problem limiting the development of *L. argentimaculatus* culture. Bonlipatanon (1988) and Emata et al. (1994) reported that two critical periods were observed: days 3-5 during the transition from endogenous to exogenous feeding, and days 18-20 during the appearance of the elongated dorsal spines. Rearing of John's snapper, *L. johnii*, larvae also revealed two critical periods at similar stages (Lim et al., 1985). However, survival of *L. argentimaculatus* larvae in our study declined gradually until day 20. Watanabe et al. (1998) reported similar results for mutton snapper, *L. analis*. Sibling cannibalism among different sized larvae contributes to mortality after day 26. No optimal procedure for avoiding cannibalism is yet known. Future studies must be planned to avoid mass mortality of larvae at these stages.

There are strong similarities in egg and larvae development between lutjanid species reared in comparable conditions (Table 2). *L. stellatus* has bigger eggs and hatched larvae than other lutjanids. The minor differences in larval development, e.g., in yolk-sac absorption and start of feeding, probably relate to differences in rearing methods and environmental conditions. The similarities of early life histories of various lutjanids will help to understand the lutjanid species. Although results with other snapper species are promising, a protocol for reliably producing juveniles has yet to be established, and techniques are far behind those for *L. argentimaculatus*.

In conclusion, *L. argentimaculatus* were spawned naturally in captivity and reared to the juvenile stage in the hatchery. S-type rotifers were a suitable initial diet. After the successful propagation and completion of its life cycle in captivity, rearing techniques for *L. argentimaculatus* have been somewhat established. However, many questions, especially about nutritional requirements, prevention of cannibalism and optimum stocking density, have yet to be answered.

Table 2. Comparison of the larval development of members of the Lutjanidae family.

	Water temperature at spawning (°C)	*Size of egg (mm)*	*Size of globule (mm)*	*Water temperature at hatching (°C)*	*Hatching time (h)*	*Size at hatch (mm)*	*Size at 3 days (mm)*	*Size at 10 days (mm)*	*Mouth opened (day)*	*Feeding started (day)*	*Yolk sac absorbed (day)*	*Metamorphosis (mm)*
L. kasmira[a]	19.8-27.6	0.78-0.85	0.13-0.14	24.8-26.2	18	1.83	3.2	-	2	3	3	-
L. campechanus[b]	23-27	0.77-0.85	0.15-0.19	23-28	20-27	1.5-2.2	2.5	2.7	3	3	3	-
L. russelli[c]	-	0.70-0.78	0.15-0.16	23.2-24.2	29	2.0-2.2	3.2	-	2-3	3	3	-
L. johnii[d]	28-31	0.77-0.85	0.15-0.17	27.5-28.0	17-18	1.6-1.7	2.73	3.0	3	3	2-3	15-20
L. stellatus[e]	24.1-26.2	0.80-0.85	0.16-0.17	23.5-24.5	30	2.5-2.6	3.4	-	2-3	3	3-4	-
L. analis[f]	28.5	0.73-0.88	-	27.7	17	-	2.76	-	-	-	-	-
L. argentimaculatus[g]	26.0-31.5	0.74-0.81	0.14-0.16	25.8-28.7	16-22	1.6-1.9	3.1	4.2	2-3	3	3-4	10.5-17.2

[a] Suzuki and Hioki, 1979
[b] Arnold et al., 1978; Rabalais et al., 1980; Minton et al., 1983
[c] Liu and Hu, 1980
[d] Lim et al., 1985
[e] Hamamoto et al., 1992
[f] Watanabe et al., 1998
[g] Present study

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