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Preliminary Study of Seed Production of the Micronesian Mud Crab *Scylla serrata* (Crustacea: Portunidae) in Korea

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Abstract : Seventeen females of the mud crab *Scylla serrata*, from the State of Kosrae, Micronesia, were transported to the Fisheries Resources Research Institute, Gyeongsangnam-do, in oxygen-filled plastic bags. After acclimatization to a 30°C holding temperature, nine females were selected for seed production trials. Spawning was hastened using eyestalk ablations; however, this may not be required in commercial-scale mud crab seed production. Primary spawning produced an average of 2.4 million hatched larvae, whereas secondary spawning produced 0.4 million. About 10 days elapsed between spawning and hatching and 30 days between hatching and crablet. Mass mortalities up to 90% were observed between stages zoea 1 and zoea 2 in every trial. The highest survival rate from zoea 1 to crablet was estimated at 0.25%. Most commercial shrimp hatcheries in Korea are equipped with almost all necessary facilities and could be converted easily to mud crab hatcheries, able to run three to four times per year using hatchery technologies developed for blue crabs and Chinese mitten crabs.

Key words : mud crab, eyestalk ablation, seed production, crablet, Micronesia

1. Introduction

Mud crabs of the genus *Scylla* are important marine resources for islanders and coastal dwellers throughout the Pacific and Indian Oceans. Four species of mud crabs, *Sylla serrata*, *S. paramamosain*, *S. olivacea*, and *S. tranquebarica*, are commercially important. Among these, *S. serrata* has the highest market value. It is distributed widely from the eastern coast of Africa to the southeastern coast of China, and from the middle part of the Australian coast to the southern part of the Japanese coast. Kim (1973) has reported the presence of *S. serrata* at Geoje-do, on the southern coast of Korea, on the basis of a young specimen caught by Kajima (1941). However, recent studies (Hong 2006) have suggested that the mud crabs distributed in Korea are not *S. serrata* but *S. paramamosain*, sensu Keenan et al. (1998).

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In 2007, the annual harvest by catch and aquaculture of mud crabs, which are associated with mangrove complexes, reached about 32,000 and 118,000 tons, respectively (FAO 2009). Demand for mud crabs in international markets is increasing steadily (SPC 2009); however, the supply of mud crabs destined for international markets is decreasing in most Pacific nations due to significant domestic demands. Studies of the commercial production of *S. serrata* crablets (seed crabs) have been ongoing for four decades (Ong 1964; Brick 1974; Hill 1974; Chen 1976; Heasman and Fielder 1983; Zeng and Li 1992; William et al. 1999; Nurdiani and Zeng 2007). However, a commercially viable larval rearing protocol has yet to be established (Ruscoe et al. 2004; Zeng and Rabbani 2005). Present mud crab aquaculture relies merely on the grow-out and fattening of wild juvenile crabs in domestic facilities. This practice will eventually threaten natural stocks, and the securing of juvenile crabs will become increasingly difficult. Some progress in the sustainable seed production of mud crabs has been achieved in

Australia, China and the Philippines, but to date, commercial scale aquaculture is still not viable.

However, *S. serrata* grows very fast in captive conditions. Crablets weighing about 10 g (carapace width: 4 to 5 cm) can grow to 400 g (carapace width: 15 cm), which is a marketable size, within 6 months with an optimum temperature regimen and an adequate food supply (Prinpanapong and Youngwanichsaed 1991, Hong 1999). Thus, mud crabs are potentially an excellent new aquaculture species during the warm season in Korea that would ease the demands on blue crabs and provide an alternative to the importation of high-value crustaceans such as king crab, lobster, and crayfish.

The major objective of this study is to provide general information on the seed production of *S. serrata*, which is essential for the establishment of a mud crab aquaculture industry in Korea. The study was focused on the sequence of larval development in the crab.

2. Materials and methods

Collection and transportation of mother crabs

Females of the mud crabs, *Scylla serrata* were caught by hand from a mangrove swamp in Utwa Village, Kosrae, Federated States of Micronesia (Fig. 1, Plate I-1) from August 4 to 6, 2006, and kept in a concrete tank with shelters. In the evening of August 7, 17 of the most active crabs with carapace widths exceeding 12 cm were chosen as the brood stock, and each of them was put into a mesh bag after washing and kept in a semi-soaked condition overnight. In the morning of August 8, each mesh bag was put into a paper bag before being placed in a 40-L oxygen bag, to eliminate completely the possibility of the crabs puncturing the oxygen bags. Then, a wet towel was put into each oxygen bag to maintain the moisture level, and the bag was filled with pure oxygen (Plate I-2). Three oxygen bags were placed together into an insulated Styrofoam box with some gel ice packs and transported to Incheon Airport by air. Upon arrival at the airport early in the morning on August 9, the mesh bags were removed and soaked in seawater before being placed into a carton with breathing holes. These were then transported to the Fisheries Resources Research Institute (FRRI), in Gyeongsangnam-do. Of the 17 crabs introduced to Korea, one died and six lost one or two appendages. The total process took about 30 hours and the crabs were kept in the oxygen bags for about 23 hours.

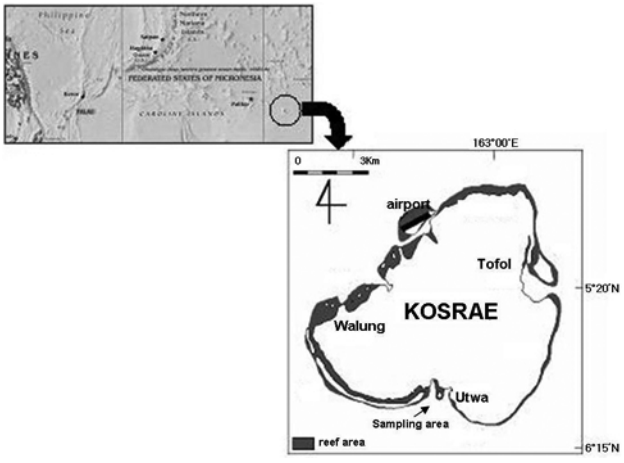


Fig. 1. Collection site of *S. serrata* in Kosrae, FSM.

Acclimatization and spawning

Upon arrival at FRRI, each crab was put in a plastic basket (42×65×35 cm) and placed in a concrete tank (5×5×1 m) with filtered seawater supply nozzles for each plastic basket. Seawater was pumped from coastal waters of the FRRI and passed through a sand and active-carbon filter. Salinity was regulated at 34 psu using natural salt to provide the crabs with salinity conditions similar to those of where they were collected.

Holding water was disinfected using ultraviolet light and was supplied through the nozzles to the baskets. The initial water temperature was set to 26°C in accordance with the local air temperature during ground transportation. Water temperature was then increased by 1°C per day until it reached 30°C, such that the basic environmental conditions were equal to those of the original habitat of the crabs. Once the predesignated temperature was reached on August 13, the nine most active crabs were chosen, and their ovarian maturity was determined by examining, in the dark by using a flashlight, the contours of their ovaries. The carapace cavity is filled by an ovarian mass in fully matured females and cannot be penetrated by light from a flashlight. Females with ovarian contours exceeding three quarters of their carapace width were considered to be mature, whereas those with ovarian contours exceeding three fifths, but not exceeding three quarters, of the width were considered premature. Six mature and three premature females were identified, and these were fed raw fish twice a day until spawning. After two hours for feeding, the uneaten food was removed from the tanks.

Induced spawning

Among the six mature females, three were randomly selected and the left eyestalk of one individual was ablated on August 13. The other two individuals underwent eyestalk

ablation on the following day. Before placing each female crab into a spawning container (130×85×50 cm), fine sand was layered into the containers, for a sediment depth of 15 cm. Spawning containers were put into a 20-ton concrete tank (10×10×2 m). The water level in the tank was maintained at 40 cm and the water temperature was maintained near to 30°C using a thermostatic heat pump. Filtered seawater was supplied to each container through a pipeline laid on the tank wall. Water exchange rates in the tanks and containers were 4 and 10 times per day, respectively. In this manner, the water temperature in each container was maintained at 29.6±0.5°C during the study.

The crabs were checked every day and spawners were collected and put into new containers, as described above. A few berried eggs were dissected daily using forceps, and their embryonic development was observed under a stereomicroscope.

Hatching and larval nursing

Eight days after spawning, when the color of berried eggs changed from yellow to dark brown, the crabs were moved into the hatching tank (5×5×1 m). The water depth in the tank was first maintained at 50 cm and increased to 1 m as hatching approached. Just before hatching, as determined by active movement of eyed embryos (Plate I-5), *Nannocropsis oculata* was added (for a total of 106 cells/m*l*) and the oxygen concentration was maintained at saturation with a liquid oxygen supply and vigorous aeration.

Hatching occurred at night. The total number of hatched larvae was determined by counting the number of larvae in five 10 m*l* subsamples which were taken from different parts of the hatching tank under dark conditions. Then, using a siphon, about one million floating larvae were transferred into a nursing tank (5×5×1 m) with a flow

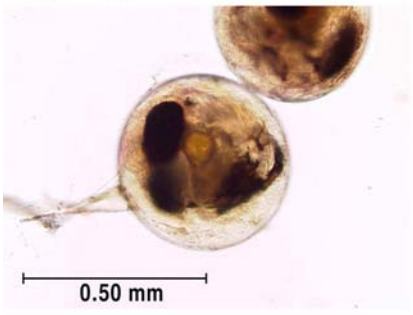
Plate I

1. *S. serrata* in a feeding ground at night.

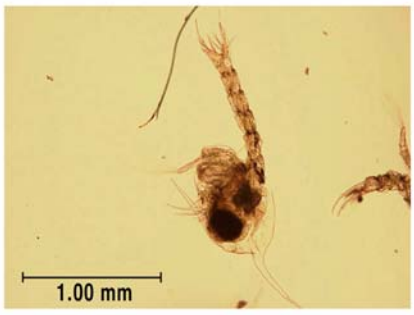
2. Packing a female *S. serrata* into an oxygen bag.

3. Spawning posture of a female *S. serrata*.

4. A berried *S. serrata*, close to the time of hatching.



5. Eyed eggs of *S. serrata*, 10 days after spawning.



6. A zoea 1 stage larva of *S. serrata*.

through water supply system. The tank was conditioned with 10^6 cells/m*l* of *N. oculata* a day to provide food to rotifers and to keep the water quality stable. The density of *N. oculara* was maintained until the larvae entered the megalopa stage. Water circulation rates in the nursing tanks were one and two times per day until the zoea 5 and megalopa stages, respectively.

Hatched larvae were fed with DHA-enriched rotifers until the zoea 5 stage. Commercial compound shrimp diets were added, beginning at the zoea 2 stage, and DHA-enriched *Artemia* nauplii were supplied beginning at the zoea 3 stage. *Artemia* nauplii and minced shellfish were provided from the megalopa stage. The density of live feed in the nursing tanks was adjusted four times a day, to 25 individuals/m*l* for the rotifers and 2 to 3 individuals/m*l* for the *Artemia* nauplii. Once a day, in the morning, about 10% of the nursing-tank water was removed through a siphon from the bottom of each nursing tank to remove dead larvae and food remnants.

At the end of the zoea 5 stage, plastic shade films and 0.5-mm net pans (30×50 cm) were placed in nursing tanks to prevent cannibalism.

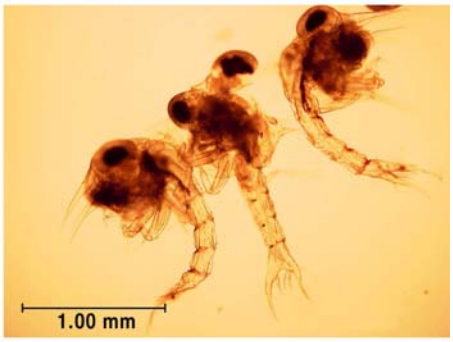
3. Results

Spawning and berring

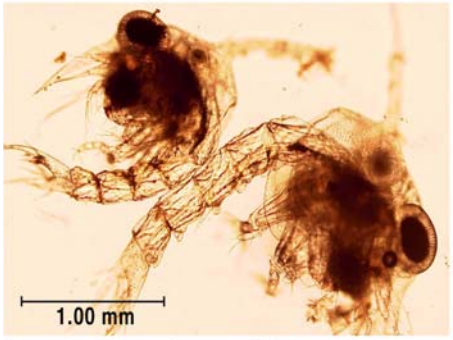
Near the time of spawning, mature female crabs hollowed the substrate and dug a shallow divot (Plate I-3) for ease of berring (attachment of spawned eggs to pleopods). Spawning was conducted during dark periods and berring

Plate II

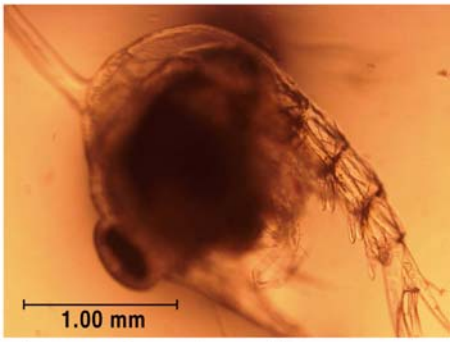
1. A zoea 2 stage larva of *S. serrata*.



2. Zoea 3 stage larvae of *S. serrata*.

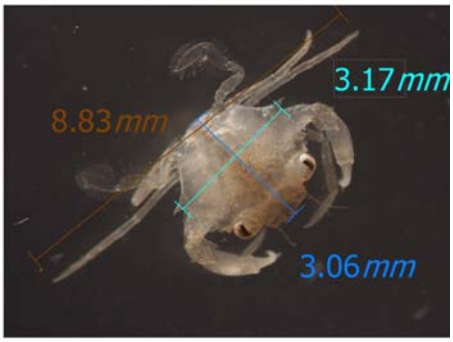


3. Zoea 4 stage larvae of *S. serrata*.



4. A zoea 5 stage larva of *S. serrata*.

5. Megalopa larvae of *S. serrata*.



6. A crab 1 stage of *S. serrata*.

Table 1. Spawning of *S. serrata* introduced from Kosrae, FSM (2006)

No.	Physical condition	Maturity*	Eyestalk ablation	Spawning		Berring
				1st	2nd**	
1	-rwl1	>3/4	Aug. 13	Sept. 2		normal
2	normal	>3/4.	Aug. 14	Sept. 2		normal
3	normal	>3/4	Aug. 14	Sept. 2	Oct. 22	normal
4	-rcp	>3/4	none	Sept. 5	Oct. 30	normal
5	normal	>3/4	none	Sept. 10		normal
6	normal	>3/4	none	Sept. 18		died
7	normal	>3/5	none	Sept. 30		noraml
8	normal	>3/5	none	Oct. 4		normal
9	normal	>3/5	none			died

*: ovarian contour/carapace width, **: without extra eyestalk ablation, -rwl1: lost 1st walking leg, -rcp: lost right cheliped.

took place immediately. The three females with ablated eyestalks spawned 20 days after ablation, whereas two intact females spawned after 23 and 28 days of holding. One intact mature female spawned after 36 days of holding but was unable to berry and died. Two females, including one with an ablated eyestalk, conducted secondary spawning on the 50th and 55th day after primary spawning (Table 1). One premature female died without spawning, but the other two premature females spawned and berried successfully after 48 and 52 days of holding.

The average number of hatched larvae in a primary spawning event was about 2.4 million; that of a secondary spawning event was 0.4 million.

Hatching and larval rearing

Immediately after berring, the mud crabs' eggs were orange, gradually turning dark closer to hatching and becoming almost black by the time of hatching (Plate I-4). Eye spots became visible from day seven. The rotation of eyed embryos were observable from day 8; embryos rotated actively as hatching drew near (Plate I-5). A total of 10 to 11 days elapsed between berring and hatching at a temperature of 29.6±0.5°C in captivity, except for the crab that spawned on September 5, for which the interval was 9 days (Table 2).

Table 2. Hatching of *S. serrata* introduced from Kosrae, FSM (2006)

No.	Spawning date	Hatching date	Incubation period (days)	No. of larvae (million)
1, 2, 3	Sept. 2	Sept. 13	11	2.5
4	Sept. 5	Sept. 15	10	2.4
5	Sept. 10	Sept. 19	9	2.4
7	Sept. 30	Oct. 10	10	2.4
8	Oct. 4	Oct. 14	10	2.4

Newly hatched mud crab larvae (zoea 1) were about 1.3 mm in length (Plate I-6). The first molt occurred two days after hatching and larvae became zoea 2 larvae (Plate II-1). They then molted at intervals of about three days before becoming zoea 5 larvae (Plates II-2, -3, and -4 and Table 3). The average rate of increase in body length during the zoeal stage was about 30% per molt. Zoea 5 larvae molted to become megalopa larvae within 7 days (Plate II-5). The color of the megalopa larvae darkened gradually as the molt proceeded, and megalopa became young crabs within 7 days (Plate II-6). The average carapace width and height of the crab 1 stage was 3.2 mm and 3.1 mm, respectively. The crab 1 stage needed two additional molts to become a crablet (seed crab) with carapace length exceeding 25 mm. On average, it took about 30 days from the time of hatching to crablet emergence.

Mortality was about 90% between the zoea 1 and zoea

Table 3. Time requirements for the metamorphosis of *S. serrata* larvae

Stage*	Z-1	Z-2	Z-3	Z-4	Z-5	Meg.	C-1	C-2	C-3
Size (mm)**	1.33	1.63	2.09	3.05	4.06	1.79	3.06	5.02	25.80
Age (day)	2	4	6	9	12	15	22	26	29

*Z: zoea, Meg: megalopa, C: crab, **zoea stage: body length, Megalopa and crab: carapace width.

2 stages and about 80% between zoea 2 and zoea 3 stages. After zoea 3, mortality dropped to around 50% for each molt. The highest survival rate from zoea 1 to crablet was 0.25% and about 2,500 crablets were produced. The possible survival rate, as estimated from the highest survival rate of each larval stage during the study, was 0.8%.

4. Discussion

Eyestalk ablation has been a classic method for promoting the molting and spawning of crustaceans since Panouse (1943) and has been widely used in commercial shrimp hatcheries owing to its effectiveness in inducing sexual maturity (Arnstein and Beard 1975; Brady and Lawrence 1992; Hansford and Marsden 1995; Mann et al. 1999; Millamera and Quinitio 2000; Zeng 2007). Ablation also affects many physiological processes in adults and developing larvae (Grossl and Knowton 2002). However, a female mud crab can retain spermatozoa delivered by a male in her seminal receptacles for up to several weeks and can use them to fertilize multiple clutches of eggs (Chen 1976). Thus, it seems that eyestalk ablation may not be required to promote sexual maturation, except when there is an urgent need for larvae, as noted by Mann et al. (1999).

Among the nine female crabs used in this study, seven produced normal batches of larvae and the average batch size was about 2.4 million. The true average fecundity of the crabs probably exceeds 2.5 million eggs, however, considering that eggs that had not hatched by midnight of the day of hatching were not counted in this study. Two cases of secondary spawning were observed, with small batch sizes of approximately 0.4 million larvae. These batch sizes were within the normal range for spawning (Mann et al. 1999; Quinito et al. 2001) but were too small to be of use in a commercial hatchery. This poor result seemed to be caused by human disturbances, an unfamiliar captive environment and poor nutrients, as pointed out by Zeng and Li (1999), Djunaidah et al. (2003) and Rabbani and Zeng (2005).

The elapsed time from spawning to hatching was slightly less than that found by Phelan and Grubert (2007) and Nghia et al. (2007), seemingly because of a slightly higher seawater temperature in this study. Pre-zoea larvae (Mann et al. 1999; Williams et al. 1999) were counted as zoea 1 larvae, and zoea 6 larvae (Zeng et al. 2004) were not observed in the present study.

Mortality between the zoea 1 and zoea 2 stages was 90%, dropping to 80% between the zoea 2 and zoea 3 stages. Mortality then dropped to 50% for the remaining molts. Such mass mortalities are not uncommon in the larval culture of crustaceans including *S. serrata* (Quinitio et al. 2001; Hamasaki et al. 2002). The density of live food and its nutritional quality (Li et al. 1999; Williams et al. 1999; Quinito et al. 2001) and temperature (Li et al. 1999; Hamasaki et al. 2002) are believed to be responsible for such mass mortality.

The present study was conducted to establish a hatchery protocol. Each experiment used the number of larvae of each developmental stage that could be easily accommodated. The highest survival rate from zoea-1 to crablet was 0.25%, but the maximum survival rate, as estimated from the highest survival rate in each larval stage during the study, was 0.8%.

Most commercial shrimp hatcheries in Korea are equipped with almost all necessary facilities for mud crab seed production and could be converted easily to mud crab hatcheries. Moreover, technologies developed for the blue crab, *Portunus trituberculatus* (Chang 2002; Kim 2006), and the Chinese mitten crab, *Eriocheir sinensis* (KORDI 1992, 1994), can also be applied to mud crab hatcheries. The above mentioned survival rate is a goal for future studies, and the recommended production scale for the first mud crab hatchery is about 100,000 crablets. At least three runs would be possible in a year and five mature females would be required for each trial.

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