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Seed Production of Mud Crab *Scylla serrata* Juveniles

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Abstract

A protocol for the large-scale rearing of the mud crab *Scylla serrata* juveniles was developed based on the results of small-scale experiments on feeding and water management. This paper also reports the success in producing the second generation (F_2) crabs.

Pond-reared adult *S. serrata* held in 10 m^3 concrete tanks with sand substrates were given fish, mussel, annelids and formulated diet. The zoeae produced were stocked in 1.5 or 10 m^3 tanks at 30 to 50 ind·l^{-1} and fed 10 to 15 *Brachionus rotundiformis*·ml^{-1}, 1 to 5 *Artemia salina* nauplii·ml^{-1} and 1.5 to 2.0 g shrimp larval commercial diet·m^{-3}·day. Water was replaced daily at 30 to 50% of the total volume starting day 3. Megalops were nursed until crab stage either in tanks or in net cages installed in ponds. Crabs were fed mussel or small shrimps (*Acetes* sp).

Hatching occurred 6 to 12 days after spawning at 26.5 to 30.5°C. A female produced 0.42 to 5.23 x 10^6 zoeae at a time. Mean survival rate from zoea 1 to 3- to 5-day old megalopa was 2.6 ± 0.8% and 32.8 ± 4.8% from megalopa to crab stage. The development from zoea 1 to megalopa required 16 to 18 days. Cannibalism and luminescent bacteria were identified as the major causes of mortality. Highest mortality was observed during the metamorphosis from zoea 5 to megalopa and megalopa to crab 1. First crab stage was obtained 23 to 25 days after hatching. Sorting the crabs during the nursery period minimized cannibalism.

Completion of the cycle in captivity was attained in 1997 and 1999 when spawns from pond-reared crabs grew to become the second-generation broodstock. The results point to a minimum age of 7.5 to 9 months at which *S. serrata* hatched their eggs after rearing from zoea 1.

Introduction

The continuous collection of wild seeds for grow-out culture due to the expanding export market for mud crab and the search for alternative culture species for shrimps has threatened the wild stock population. Of the various mud crab species, *S. serrata* (=*S. oceanica*, Estampador 1949) commands a higher price in the export market due to its excellent meat quality and large size.

In an attempt to culture larvae of *S. serrata*, studies on water quality, phytoplankton and food organisms (Brick 1974), use of recirculating system (Heasman and Fielder 1983), amount and different diet combinations

(Baylon and Failaman 1999; Quinitio et al. 1999; Williams et al. 1999; Zeng and Li 1999), and salinity tolerance (Parado-Estepa and Quinitio 1999) have been conducted but only on a small-scale. The effect of diet on *S. serrata* broodstock (Millamena and Quinitio 2000) has been initiated on a large-scale. To develop a reliable technology for consistent spawning and production of crab juveniles, research efforts should focus on large-scale rearing of mud crab. An understanding of the mud crab's life cycle in captivity is equally important.

This paper describes the protocol developed for the large-scale rearing of *S. serrata* juveniles based on the best management and feeding schemes defined in small-scale experiments. This paper also reports the success in producing the next generation of adult crabs, thereby, completing the life cycle of *S. serrata* in captivity. The taxonomic classification of the species was based on Keenan et al. (1998).

Materials and Methods

Breeding

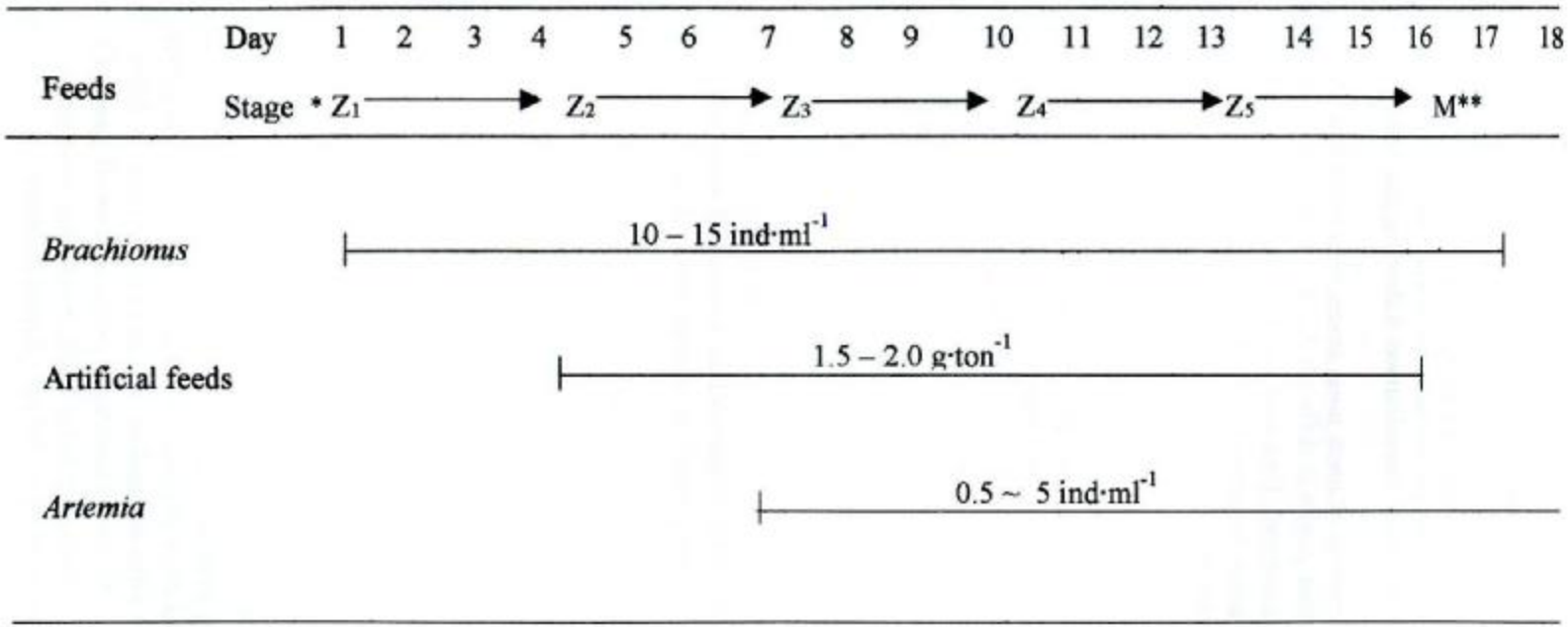
Eight to ten pond-reared adult *S. serrata* females (313 to 950 g body weight, BW; 13.0 to 17.4 cm carapace width, CW; 8.5 to 11.8 cm carapace length, CL) were obtained from crab dealers every four months and subjected to unilateral eyestalk ablation. Crabs were held in a 10 m^3 circular concrete tank with sand substrate and shelters. The tank was covered to minimize disturbance. Food consisted of SEAFDEC-formulated diet (Millamena and Quinitio 2000) given at 2% BW in the morning and fish or mussel given at 10% BW in the afternoon. Live marine annelids were offered to crabs once every one to two weeks. Water in the tank was changed daily prior to feeding. Sampling for egg-carrying females (berried) was done twice a week. Berried females were disinfected with 150 ppm formaldehyde and held individually in 300 l or 1.5 m^3 fiberglass tanks with aerated seawater (32 ppt) until the eggs hatched.

Seawater supply for crab breeders and larvae were chlorinated in the reservoir with 10 to 20 ppm calcium hypochlorite and neutralized with sodium thiosulfate after 12 to 24 h.

Larval rearing

Harvesting and estimation of newly-hatched zoeae were done through aliquot sampling. Zoeae stocked in 1.5 and 10 m^3 circular tanks at 30 to 50 ind·l^{-1} were given the rotifer *Brachionus rotundiformis* at 10 to 15 ind·ml^{-1} (Table 1). The phytoplankton *Chlorella* at 50,000 cells·ml^{-1} was added as food for rotifers and for water conditioning. Shrimp larval diet was fed beginning late zoea 1 following the method of Quinitio et al. (1999). At zoea 3, newly hatched *Artemia salina* were given at 1 to 3 ind·ml^{-1}, and increased to about 5 ind·ml^{-1} as the larvae developed to megalopa. Starting at

Table 1. Feeding scheme used in the culture of *Scylla serrata* larvae



*Z- Zoea, **M - megalopa

day 3 about 30% of the water was replaced daily and increased to 50% as larval development advanced. Dead larvae and uneaten feeds were siphoned out prior to water change. Ambient salinity and temperature ranged from 32 to 34 ppt and 26 to 30.5ºC, respectively. Larvae were exposed to a natural photoperiod of about 11 to 12 h light/13 to 12 h dark.

Before water change, zoeae and water samples were taken daily in some tanks for microbial examination following the method of Lavilla-Pitogo et al. (1990).

Aliquot sampling of each zoea stage was tried but the aggregating behavior of the zoea made it difficult for the researchers to get a good estimate of the population. However, actual counts were done for 3- to 5-day old megalops prior to transfer to 10·m^3 concrete tanks or net cages in brackishwater ponds.

Nursery

Megalops were stocked at 1,000 ind·m^3 in concrete tanks. Food consisted of newly hatched *Artemia* at 3 to 5 ind·m^{-1} or adult *Artemia*, and shifted to minced trash fish, green mussel, or *Acetes ad libitum* twice daily as soon as megalopa metamorphosed to crab stage. *Chlorella* was added to the tanks throughout the culture period. Excess feeds were siphoned out prior to feeding and seawater (28 to 30 ppt) was replaced at 30 to 50% daily. PVC pipe cuttings, black nets and occasionally, seaweed *Gracilariopsis bailinae* were distributed in all the tanks as shelters. The culture of megalops in net cages was done following Rodriguez et al. (this volume).

Salinity trial

Three- to five-day old megalops (mean BW = 8.9 mg; CW = 2.78 mm) reared in 1.5 m^3 tanks at 1500 ind·tank were exposed to either 26 ± 1 ppt or ambient salinity of 32 ppt for 18 days. Each treatment was replicated five times. All other culture conditions were similar to those described in the Larval Rearing section.

Sorting

The survival rate from megalopa to 4- to 7-day old crab was 49.5 ± 3.0% and further decreased to 28.8 ± 5.2% after another 10 to 14 days in tanks due to cannibalism. To reduce the chances for cannibalism, the effect of size-grading of stock was determined. Sibling hatchery-reared crabs (C10 or 35 days from hatching) were sorted into two size groups, the large (0.04 ± 0.001 g BW, 0.63 ± 0.01 cm CW) and the small (0.015 ± 0.0004 g BW, 0.40 ± 0.005 cm CW). These were stocked at 400 ind·1.0 m^3 tank as follows: Treatment L - large size only, M - combination of large and small sizes (200 crabs in each group), and S - small size only. Each treatment was replicated four times. Salinity was maintained at 26 ppt. Equal numbers of black net substrates

were distributed in all the tanks. Water and feeding management were similar to those described in the Nursery section. After 10 days, the mass weights of crabs in each tank were determined. Crabs were then sorted into large, medium, and small size groups. Crabs in each size were weighed and the CW and CL measured using a vernier caliper after counting.

Completion of the life cycle

Wild juveniles from Leyte, Eastern Philippines (7 to 11 g BW) were cultured in brackishwater ponds and grown to broodstock size (400 to 500 g BW) in 1997. The juveniles produced by these broodstock were cultured in two 200 m^2 compartments in Molo, Iloilo, Western Philippines. Pond preparation and fertilization were done following Triño et al. (1999). Net enclosures were installed along the perimeter of pond dikes to prevent escape of crabs. One compartment was stocked with 57 crabs (81.3 g BW) from one family and the other with 128 crabs (14.8 g BW) from another family. Food consisted of trash fish and mussel given alternately at 10% BW daily. After four months, six females of 340 to 580 g BW (11.3 to 15.0 cm CW) were ablated and held in maturation tanks until spawning.

A better result on the closing of the life cycle was obtained from pond-reared adult crabs sourced from Samar, Eastern Philippines (885 g BW, 17.6 cm CW) and Negros Occidental, Central Philippines (460 g BW, 13.9 cm CW) in May and July 1999, respectively. The second-generation broodstock produced in April 2000 in the Leganes ponds, Iloilo, spawned and hatched viable zoeae in June 2000 that were subsequently reared until the juvenile stage at the SEAFDEC/AQD ponds in Dumangas, Iloilo. Marketable-size crabs were harvested in December 2000. Breeding, larval and nursery rearing protocols were similar to those previously described.

Statistical analysis

Data on survival and body measurements were analyzed using analysis of variance followed by Duncan's new multiple range test (Walpole 1982; Gomez and Gomez 1984). All percentage and body weight data were transformed to arcsin or log values, respectively.

Results

Breeding and larval rearing

The results were based on production runs done from March to December 1998. Of the 33 crab breeders obtained, 17 spawned once, 11 spawned twice and 5 spawned thrice. However, only 25 crabs produced viable larvae. The eggs of the remaining crabs were aborted due to either lack of fertilization or fungal infestation. Hatching occurred 6 to 12 days following spawning at 26.5 to 30.5ºC. A female produced 0.42 to 5.23 $x10^6$ zoeae at a time.

Of the 25 runs conducted (one run = rearing of zoeae from one female), eight runs were discarded. The survival rate from zoea l to megalopa reared in 1.5 and 10 m^3 tanks showed no significant difference hence, means were pooled. Zoea 1 reared to three- to five-day old megalopa had a mean survival rate of 2.7 ± 0.8% and 32.8 ± 4.8% from megalopa to crab stage. The duration from zoea to megalopa ranged from 16 to 18 days. Two larval stages were observed in the same tank indicating asynchronous molting. About 10 to 20% of the larval population attained the megalopa stage 16 to 17 days after hatching and about 50% were in the megalopa stage after 17 to 18 days. All larvae were in the megalopa stage on days 18 to 19. First crab stage was obtained 23 to 25 days after hatching. The bacterial profile of two larval rearing runs is shown in figure 1. Luminescent bacteria in the water were detected starting at day 2 with a population of 10^1 cfu·ml^{-1} increasing to 10^3 cfu·ml^{-1} as the culture progressed. The increase of luminescent bacteria in the larvae was apparent starting days 9 and 10 (10^3 cfu·ml^{-1}). Presumptive *Vibrio* remained high

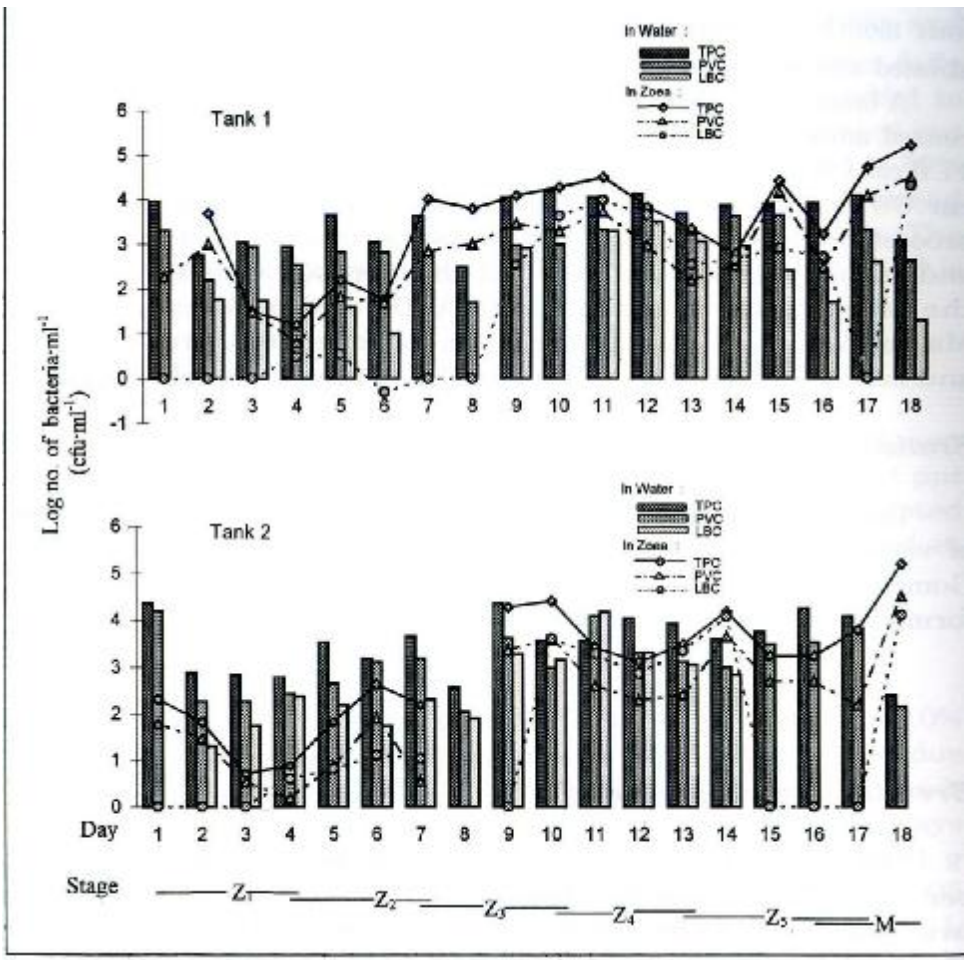


Fig. 1. Bacterial profile of rearing water and *Scylla serrata* larvae in tanks. Counts obtained from nutrient agar and TCBS medium are expressed in colony-forming units (cfu) per ml. Z - zoea; TPC - total plate count; PVC - presumptive *Vibrio* count; LBC – luminous bacteria count

from days 9 to 18. Although some dead larvae were observed during this period, a significant decrease in the population was noted from zoea 5 to megalopa.

Salinity trial

Megalopa survived better to crab stage in 26 ± 1 ppt (40.0 ± 4.5%) than in 34 ppt (26.2 ± 2.0%) ($P<0.05$). However, growth rates did not differ between the two treatments (Table 2).

Sorting

After 10 days, the survival rate of crabs stocked at a small and uniform size (S) had significantly higher survival (86.8 ± 2.9%) than the two-size group (M) (68.1 ± 4.7%) but not with the large and uniform size group (L) (77.1 ± 4.4%) (Fig. 2). As expected, mean mass weight in the L group (49.6 ± 6.2 g) was significantly higher than in the S group (27.9 ± 2.8 g). However, mean mass weight in the M group (36.8 ± 5.8 g) did not significantly differ from the S or L treatment groups (Fig. 2).

At harvest, 71.8 ± 5.7% of the crabs in the L group were large (0.3 ± 0.02 g BW) while the rest were medium size (0.10 ± 0.004 g BW) (Fig. 3). In treatment S, only 3.3 ± 1.9% of the crabs were large (0.3 ± 0.01 g BW)

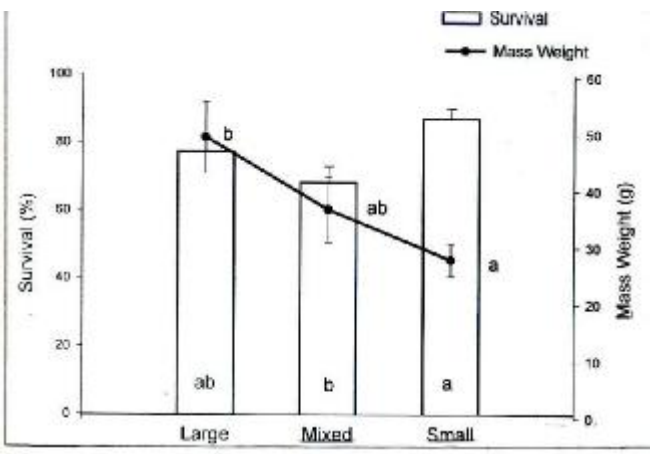


Fig. 2. Mean survival and mass weight of *Scylla serrata* crabs initially stocked in nursery tanks at uniformly large or small size, or combination of both sizes after 10 days of culture.

Table 2. Body measurements and survival of *Scylla serrata megalops* reared to crab instar (18 days culture period) at two salinity levels.

	Treatment (ppt)	Body weight^{ns} (mg)	Carapace width^{ns} (mm)	Carapace length^{ns} (mm)	Survival* (%)
Initial		8.9 ± 0.01 (10)**	2.8 ± 0.038	2.6 ± 0.04	
Final	26 - 28	40.7 ± 3.0 (20)	6.66 ± 0.18	5.1 ± 0.1	40.1 ± 4.5^{a}
	32	40.0 ± 3.0 (20)	6.45 ± 0.17	4.8 ± 0.1	26.2 ± 2.0^{b}

^{ns} No significant difference
* Significant at $P< 0.05$
** Means ± SEM (no. of samples)

and 2.9 ± 1.7% were small (0.03 ± 0.00g BW), while the remaining 93.7 ± 1.6% were medium size (0.1 ± 0.01 g BW). Crabs in treatment M were mostly medium (76.3 ± 5.8%, 0.1 ± 0.004 g BW) or large (20 ± 7.6%, 0.3 ± 0.02 g BW) and few were small (3.7 ± 2.3%, 0.03 ± 0.00 g BW).

Completion of the life cycle

Juveniles sourced from Leyte in June 1996 grew to 500 g BW after six months in ponds (Fig. 4A). In May 1997, after five months in the maturation tanks, a female spawned and hatched its eggs (335.5 x 10^3 zoeae). Zoeae were cultured and the juveniles produced in June 1997 were grown to adult size. Only one (580 g BW, 150 mm CW) of the six parental F_1 produced 200,000 viable zoeae after two months in the maturation tank or nine months after the hatchery phase (Fig. 4A). The larvae survived until zoea 5.

The completion of the life cycle of *S. serrata* from Negros Occidental and Samar is presented in Fig. 4B. The juveniles from two females (S24 and S31) attained adult size after six months in ponds. The five F_1 females (701 g mean BW, 15.08 cm CW) that were brought back to the hatchery spawned but only two females (FS5 = 620 g BW, 14.53 cm CW and FS3 = 790 g BW, 16.13 cm CW) had viable zoeae. The rest had unfertilized eggs due to the absence of mating. All the parental F_1 females were not subjected to eyestalk ablation. The performance of the offspring of P_0 and F_1 females is presented in table 3.

Discussion

Results showed that *S. serrata* eggs hatched within 6 to 12 days at 26.5 to 30.5ºC while Marichamy and Rajapackiam (1991) reported 7 to 14 days at 25 to 30ºC. The incubation period of *S. serrata* eggs at 18 to 20ºC was two to three times longer than at 26 to 28ºC (Heasman and Fielder 1983). In the present study, when incubation period was extended to more than eight days, fungal (*Lagenidium*) and ciliate (*Zoothamnium*)

Table 3. Performance of offspring of the P_0 and F_1 *S. serrata* broodstock

Broodstock no.	Incubation period (days)	Total no. of zoea (x 10^3)	Survival (%)		
			Zoea to megalopa stage	Megalopa to crab stage	
P_0					
S 31	10	1955	0.15	10	C31*
S 24	11	1100	0.6	1	C31
F_1					
FS 5	11	2700	0.67	30	C16
FS 3	9	1550	0.74	21	C9

*C = means crab stage and the number corresponds to the age of the crab (e.g., C9 means ninth day after they have metamorphosed to crab stage)

infestations were sometimes detected and caused egg mortality. Microbial fouling may cause retarded embryonic development and egg mortality (Sadusky and Bullis 1994) due to restricted oxygen exchange across the egg membrane (Fisher 1986). *Lagenidium* infection in eggs may also lead to mortality in the newly hatched larvae (Millikin and Williams 1984). To counteract fungal and ciliate infestations, 0.10 $mg \cdot l^{-1}$ Treflan (44% trifuralin) was administered every two days in the hatching tank in the succeeding runs. This treatment had no detrimental effect on the eggs and newly hatched zoeae. Trifuralin at 0.003 $mg \cdot l^{-1}$ was a useful treatment for fungal mycosis in the giant crab *Pseudocarcinus gigas* larvae and had no chronic toxic effects (Gardner and Northam 1997).

Luminescent bacteria are generally found in the sea (Ruby and Nealson 1978) and may harm the larvae especially when they are exposed to stressful conditions. The pathogenicity of luminescent bacteria *Vibrio harveyi* on *S. serrata* larvae was high as opposed to *V. carchariae*, *V. alginolyticus* and *V. parahaemolyticus* (Parenrengi et al. 1993). The mortality from day 10 onwards is somehow related to the high luminescent bacterial load in both water and larvae. In shrimp, the continuous exposure of larvae to high *Vibrio* populations in the medium can result to vibriosis, as bacteria are able to multiply on the larval surface (Lavilla-Pitogo et al. 1990). In this study, *Vibrio* apparently dominated the bacterial population as shown in the high presumptive *Vibrio* counts (PVC) as early as days 1 or 2. The onset of mortalities may be related to the increase in luminescent bacterial load in the larvae. When *Vibrio* reached 10^2 $cfu \cdot ml^{-1}$ in the rearing water, water replacement up to 80% and prophylactic treatment apparently led to the reduction of bacterial population in the water. The exposure of larvae to a high *Vibrio* population was somehow prevented in succeeding runs.

The major food items for crab larvae are *Brachionus* and *Artemia*. Live and moving animal food is preferred over plant food by crabs (Warner 1977). Larval survival and development improve with increasing density of *Brachionus* and *Artemia* in small-scale culture (Brick 1974; Heasman and Fielder 1983; Zeng and Li 1999). In the present study, *Brachionus* and *Artemia* were still present in the larval tank the following day prior to water

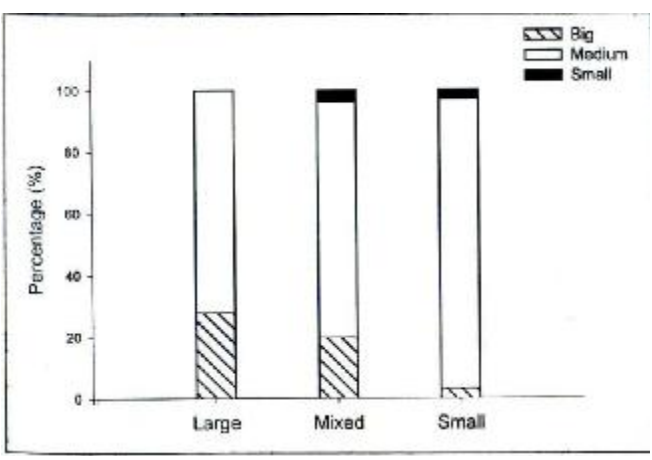


Fig. 3. Percentage of large, medium and small-sized *Scylla serrata* crabs after 10 days of culture in nursery tanks

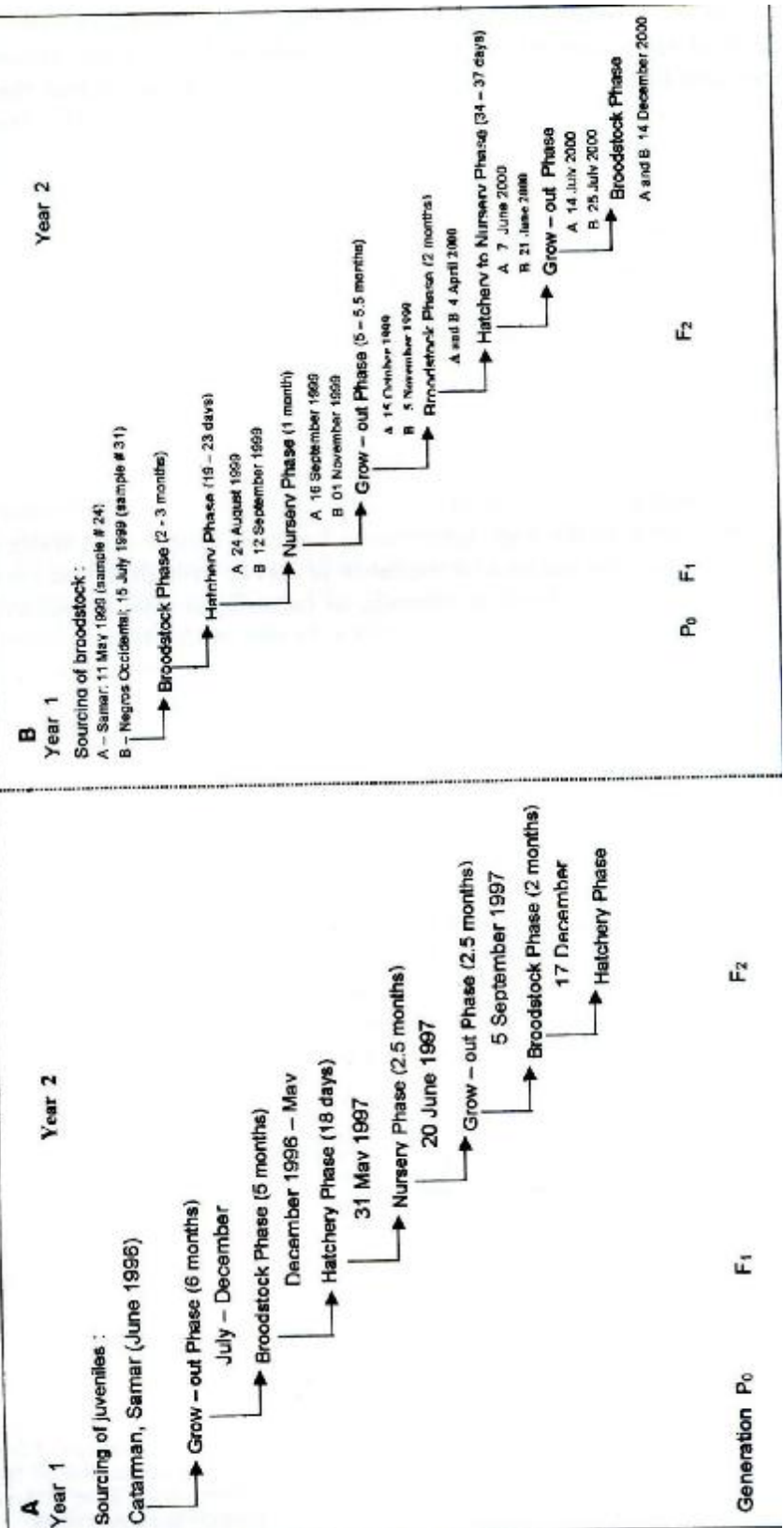


Fig. 4. Duration of culture during the completion of the life cycle of *Scylla serrata* from Samar in 1997 (A) and Samar and Negros Occidental, Philippines in 1999 (B). The dates indicate the day of stocking in each culture phase.

change and feeding. *Brachionus* counts were sometimes higher than the density maintained the previous day. In a suitable environment, an 18 h old *Brachionus* reproduces its first offspring and reproduction continues throughout its life. During peak reproductions (days 1 to 4), eggs are extruded every 4 to 6 h and hatch after another 12 h (Hoff and Snell 1993). *Brachionus* in the larval rearing tank possibly reproduced since water change and feeding of zoeae with *Brachionus* were done at least every 24 and 18 h, respectively. The requirement of zoea for *Brachionus* could also be reduced by supplementation with artificial feed. Although an increase in food density may improve larval survival, feeding *Artemia* over 5 ind·ml^{-1} may not be economical at a commercial scale. Initial economic analysis showed that *Artemia* comprised more than 50% of the variable cost.

The five zoea stages were completed 15 to 17 days after hatching. Duration of each zoea stage was 3 to 4 days and 6 to 8 days for megalopa. Marichamy and Rajapackiam (1991) reported 3 to 4 days duration for each of the 5 zoea stages and 8 to 11 days for megalopa at 27 to 30ºC. Mortality was highest during metamorphosis from zoea 5 to megalopa and from megalopa to crab 1 as many dead larvae were siphoned out from the tank bottom. These molts were accompanied by major morphological changes in the crab. The inability of zoea 5 to molt completely to megalopa was observed upon examination of the dead larvae. High mortality at zoea 5 may be related to high population of luminescent bacteria in the larvae in some of the runs. Likewise, nutritional deficiencies of the food during the zoeal stages might have delayed effects in the growth and survival of megalopa. The addition of high unsaturated fatty acids are efficient in promoting growth (Jones et al 1979) thus, inclusion of these in the diet may improve both growth and survival.

Adult *S. serrata* females migrate to more saline conditions to release their larvae (Ong 1964; Hill 1974, Robertson and Kruger 1994). The duration of *S. serrata* zoea development varies marginally over 22 to 28ºC and 30 to 35 ppt. (Ong 1964, DuPlessis 1971, Brick 1974, and Heasman and Fielder 1983. Megalopa reared to crab stage at 26 to 28 ppt and 24.5 to 27.8ºC fed *Artemia* nauplii alone had a 53% survival rate (Heasman and Fielder 1983). In the present study, survival of megalopa to crab stage at 26 to 28 ppt was better than at 34 ppt. Hence, reduction of salinity at late megalopa stage has been included in our protocol. This process also serves as the first step in the acclimatization of megalops prior to stocking in net cages in ponds where salinity ranges from 20 to 30 ppt. Crab ingression to estuaries that feature low salinity may start at the megalopa stage.

Cannibalistic behavior became apparent at zoea 4 and 5, when zoea 5 attacked the slow developing zoea 4. Asynchronous molting of crab larvae in the same tank is perhaps due to genetic difference, disease, and the individual's ability to capture the food. Although thinning out of the population may be done at late zoea stage to reduce cannibalism, the aggregating behavior of the larvae still makes them vulnerable to predation. Mortality due to cannibalism greatly increased from megalopa onwards. Pincers are developed at the megalopa stage and therefore, grasping becomes easier.

The significant decrease in population over time and size-grading of stock every 10 days resulted in the maintenance of a more homogeneous size of crabs in tanks. Likewise, trimming of the pincers once during the 30-day culture period in nursery tanks could reduce cannibalism. Since the process is tedious, its application is only practical for a small population. After one to two molts, crabs regenerated the trimmed pincers that immediately became functional. Crabs are more vulnerable to predation immediately after molting while in the soft-shell condition. Provision of sufficient nets, PVC pipe cuttings and seaweeds, *Gracilaria* as refuge somehow minimized the rate of predation. However, harvesting of crabs took longer when *Gracilaria* was used as opposed to nets and pipes. The vegetative fragments of *Gracilaria* had to be separated while the nets and pipes were simply shaken to retrieve the clinging crabs.

The reproductive performance of the first generation (F_1) captive broodstock was not as good as those initially reared from wild juveniles (Po) obtained from Samar (Table 3). The F_1 crab that released about 200 $x10^3$ zoeae was the largest (580 g BW; 15.0 cm CW) of the six breeders. However, an improvement in the reproductive performance of the F_1 broodstock obtained in 1999 was observed. This may be due to the improvement in water quality and holding conditions in grow-out ponds. The ponds utilized in the first run (1997) were adjacent to possible sources of industrial and domestic discharges while the ponds in the later runs (1999 and 2000) had better water quality.

According to Jayamanna and Jinadasa (1993), wild *S. serrata* crab attained first maturity at 12 cm CW and produced about 3 x 10^6 larvae. However, Robertson and Kruger (1994) obtained a wild mature female with only 10.4 cm CW. Prasad and Neelakantan (1989) noted a direct relationship between size and fecundity in *S. serrata* up to 14.0 cm CW. The batches of breeders used in the production runs in the present study produced 0.42 to 5.23 x 10^6 zoeae per female (13.0 to 17.0 cm CW). Size of the females is not related to fecundity. The results point to a minimum age requirement of 7.5 to 9 months at which *S. serrata* spawned after rearing from zoea 1. Ong (1966) reported that a 16^{th} instar, cultured from F_1 from a wild female, spawned 13 months after the zoea stage. Although the number of crab breeders initially used was not sufficient, it allowed us to close its life cycle that would provide information towards domestication of crabs.

Further refinement of the protocol is being done to improve survival from megalopa to early crab stage as well as to find partial replacement for *Artemia* so that the technology would become economically viable.

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