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Reproductive Performance and Larval Quality of Pond-raised *Scylla serrata* Females Fed Various Broodstock Diets

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Abstract

Scylla serrata females with initial body weight (BW) of 350 to 400 g were previously raised on a defined diet of 75% brown mussel meat and 25% fish bycatch in grow-out ponds at Molo, Iloilo City, Philippines for 120 days. Crabs were stocked in three units of 4 m diameter concrete indoor tanks at the Crustacean Broodstock Wet Laboratory of SEAFDEC Aquaculture Department in Tigbauan, Iloilo, Philippines. Tanks had sand substrates and were supplied with sand-filtered and chlorinated seawater in a continuous flow-through system with adequate aeration. Each female was provided with individual shelter. Before stocking, crabs were tagged on their carapace and half of the females were ablated while the other half remained intact. Broodstock were fed either T1, natural food consisting of mussel and fish bycatch; T2, a mixed diet of natural food and formulated diet; or T3, a formulated diet. Broodstock reproductive ability was measured as percent spawnings, spawnings with hatching, fecundity or number of eggs per g BW of female, egg fertilization rate and total zoea produced. Larval quality was measured as larval stage index or ability to attain the megalopa stage, the highest larval stage. Females fed on all dietary treatments were capable of maturation and spawnings. However, mixed diet feeding (T2) improved broodstock performance and larval quality over those fed either natural food (T1) or formulated (T3) alone. Eyestalk ablation improved fecundity and produced higher total zoea in T1 and T3 although lower in egg fertilization rate than intact females. All zoea larvae in ablated T2 females were successfully reared to megalops. Overall improvement in larval quality of both ablated and intact females compared with previous studies on wild-caught females was attributed to their dietary history in grow-out ponds. Feeding the females with a suitable diet in ponds enabled them to fortify the reserves of nutrients needed for egg development and improve stability in larval production.

Introduction

The mud crab *Scylla serrata* is a commercially important species in the Indo-Pacific countries such as Australia, Japan, Taiwan and the Philippines. In the Philippines, mud crab culture is an important source of income among small-scale fishermen in coastal communities (Laviña 1980). Currently, there is an increasing interest in crab culture as an alternative to shrimp farming.

Seed production of *S. serrata* is still dependent on spawners that are captured from the wild. Inadequate spawner supply is a major constraint to further development of the industry. Over-exploitation, environmental pollution and mangrove conversion have led to this loss (Hill 1982; Heasman and Fielder 1983). Domesticated broodstock can be a convenient alternative to wild spawners for a steady supply of the seeds and stability in the production of larvae.

The nutritional status of breeders has been known to affect reproductive performance and determines the quantity and quality of eggs and start-feeding larvae and eventually their hatchery performance (Teshima and Kanazawa 1983; Harrison 1990; Browdy 1998). This is true for crustaceans, in particular, as the lecithotropic character of their eggs requires that nutrients needed for the development of the embryo and early larval stages are available in the egg yolk. Hence, studies on the nutritional needs of pond-raised mud crab broodstock, aimed at improving the quality of their offspring, are necessary.

Our earlier study (Millamena and Quinitio 2000) on wild-caught *S. serrata* females sourced from catches by fishermen has shown the importance of broodstock nutrition to the success of crab culture through its effect on offspring quality. However, larval quality is still variable and influences hatchery success as some larval rearing runs did not reach the megalopa stage.

The objective of this study is to further examine the reproductive performance and larval quality of *S. serrata* broodstock previously cultured under known dietary regimens in brackishwater ponds. The result of this study will further contribute towards developing a technology for broodstock development and management.

Materials and Methods

Diets

Crab broodstock were fed on either natural food (T1), combination of natural food and formulated diet (T2), and formulated diet (T3). Natural food consisted of mussel meat (*Perna veridis*) and fish bycatch (*Leiognathus* sp.) given on alternate days. The formulated diet (45.03% crude protein and 11.64% lipid) is similar to that of Millamena et al. (2000), which is a modified formulation for shrimp broodstock. The diet was formulated using the fatty acid and amino acid profiles of mature crab ovaries as basis. The diet composition and proximate analysis are given in table 1.

Broodstock were gradually acclimatized to the formulated diet for 10 days by slowly withdrawing natural food and replacing it with formulated diet in increasing increments, except in T1 where natural diet were fed throughout the duration of the experiment. Daily feeding rate was 6 to 10% of BW for natural food, 2 to 3% of BW for formulated diet, and half these required amounts for the mixed diet. Feed was given thrice daily at 0800,

1300 and 1700h. Excess feed was monitored and feeding rate was adjusted accordingly.

Culture

Pond-raised premated *S. serrata* females with initial body weight of 350 to 400 g, were used as experimental animals. They were previously reared on a mixed diet of 75% brown mussel meat and 25% of fish bycatch in brackishwater ponds at Molo, Iloilo City, Philippines. Crabs were harvested then stocked in three units of 4-m diameter concrete indoor tanks at the Crustacean Broodstock Wet Laboratory of the SEAFDEC Aquaculture Department in Tigbauan, Iloilo, Philippines. To reduce cannibalism, each female was provided with a 20 cm x 20 cm x 10 cm high monoblock shelter, made of wood and black nylon net. Tanks had sand substrates, around 12 cm thick. Sand-filtered, chlorinated (20 ppm available chlorine) and neutralized seawater was supplied in a continuous flow-through system with adequate aeration. A constant water depth of 25 cm was maintained. Three experimental runs were conducted. The duration of each run was 90 days.

Before stocking, crabs were tagged on their carapace and disinfected with 100 ppm formaldehyde for one hour. Crabs were stocked at eight females per tank in three units of 10 m^3 concrete maturation tanks. Four females were eyestalk ablated and the other four were unablated. Measurement of initial egg diameter was done through ovarian biopsy to assess index of maturity at stocking. Sampling for berried crabs was done twice weekly. Spawned crabs were transferred to 300 l fibreglass tanks for egg incubation and hatching of eggs. Sampling for egg fertilization rate was conducted on the fifth and tenth day after transfer to hatching tanks. Upon hatching of eggs, the total number of zoea produced was estimated by taking six-50 ml aliquot water samples from the hatching tank. Broodstock were subsequently returned to broodstock tanks and observed for rematuration.

Broodstock reproductive ability was evaluated based on percent spawnings with hatching, fecundity or number of eggs per g body weight of female, egg fertilization and hatching rate, zoea production and quality of zoea as determined by growth index (Villegas and Kanazawa 1980).

Table 1. Composition of broodstock diet for the mud crab *Scylla serrata* (modified from Millamena et. al. 1986).

Ingredients	Percentage
Chilean fish meal	20
Shrimp head meal	20
Squid meal	20
Wheat flour	17
Seaweed (*Gracilaria* sp.)	4
Cod liver oil	5
Lecithin	3
Cholesterol	1
Vitamin mix[a]	3
Mineral mix[a]	4
Dicalcium phosphate	3

[a]vitamin and mineral mix after Kanazawa, 1981.

Proximate composition (%):

Crude protein	45.03
Crude fat	11.64
Crude fiber	5.18
Nitrogen free extract	23.13
Ash	15.02

Zoea were cultured in 250 l and 1-ton fiberglass tanks to determine the larval stage index based on the highest larval stage that was attained. The larval stage index was calculated as:

Larval stage index (LSI) = A/10

where, A = absolute value x number of larvae. Absolute values were assigned as 1, 2, 3, 4, 5, and 6 which correspond to crab larval stages starting from zoea 1 to zoea 5 designated as Z1, Z2, Z3, Z4, Z5, respectively, until the megalopa stage or Z6.

Three experimental runs were conducted from March 1998 to February 1999 with replications over time. Treatments were randomly assigned to the three tanks. Culture period was 120 days per experimental run.

Chemical analyses

Analyses for proximate composition were done on natural food and artificial diet according to Association of Official Analytical Chemists (AOAC 1984) methods. Monitoring of water quality parameters followed the procedure of Strickland and Parsons (1972). All analyses were conducted at the Centralized Analytical Laboratory of the SEAFDEC Aquaculture Department.

Water quality parameters that were monitored daily were seawater temperature and salinity. Dissolved oxygen (D0), ammonia and nitrite nitrogen were monitored thrice weekly during the 120-day culture period. These parameters were within suitable levels as specified by Chiu (1988): water temperature, 24 to 30^0C; seawater salinity, 32 to 35 ppt; ammonia nitogen, 0.1 to 0.95 ppm; nitrite nitrogen, 0.0 to 0.19 ppm; DO, 4.0 to 7.0 mg/l.

Statistical analysis

Data on reproductive performance of ablated and intact females were analyzed using one way analysis of variance (Gomez and Gomez 1984) and Duncan's multiple range test (P = 0.05) was used to test significant differences among treatment means (Duncan 1955). Arcsin transformation was used in the analyses of data in percentages. Computer software developed by Statistical Analysis Systems (SAS 1992) was used.

Results and Discussion

The relative effects of diet on the reproductive performance of intact and ablated mud crab broodstock in three trials are summarized in table 2.

Results showed that mud crab females given a combination of natural food and formulated diet (T2) gave the highest number of spawnings (24), next highest spawnings with hatching was 79%; no. of eggs per g BW females was 5,262 eggs·g^{-1} BW; total zoea production was 31,251 x 10^3; and broodstock survival was 67%.

Those that were given natural food alone (T1) had 20 spawnings with 75% hatchings; intermediate fecundity of 4,759 eggs·g^{-1} BW; total no. of zoea was 18,787 x 10^3; lowest mean egg fertilization rate of 66%; and broodstock survival was 58% over the 120-day culture period.

Broodstock given the formulated diet alone (T3) had 16 spawnings with 81% hatchings; lowest fecundity of 2,319 eggs·g^{-1} BW; total no. of zoea was 16,801 x 10^3; and broodstock survival was 55% but highest in mean egg fertilization rate at 80%.

The effect of eyestalk ablation on the responses of females fed on the dietary treatments is shown in figure 1. Intact females had better egg fertilization rates in all treatments, with higher total number of zoea produced, except in T3. However, ablated females given T1 and T3 diets were

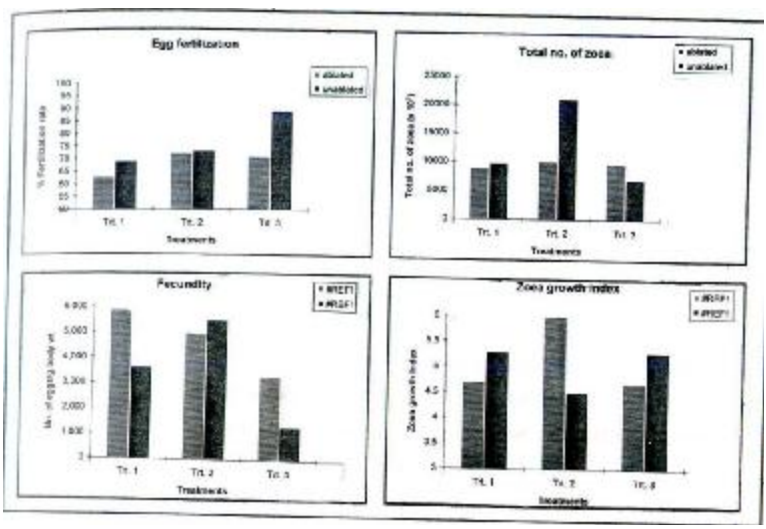

Fig. 1. Reproductive performance of pond-raised ablated and unablated mud crab *S. serrata*

Table 2. Reproductive performance and larval quality of pond-raised ablated and unablated *S. serrata* females fed various diets

Parameter	Treatment 1 natural food (NF)	Treatment 2 1:1 NF to AD	Treatment 3 artificial diet (AD)
Number of spawnings	20	24	16
with hatching	15 (75%)	19 (79%)	13 (81%)
without hatching	5 (25%)	5 (21%)	3 (19%)
Mean egg fertilization			
(%)	66	72	80
ablated	63	72	71
unablated	69	73	89
Mean no. of eggs·g^{-1}			
body weight	4,759	5,262	2,319
ablated	5,899	4,989	3,302
unablated	3,619	5,536	1,335
Total no. of zoea (x10^3)	18,787	31,251	16,801
ablated	8,977	10,067	9,766
unablated	9,810	21,184	7,035
Mean zoea growth index	5.0	5.2	5.0
ablated	4.7	6.0	4.7
unablated	5.3	4.5	5.3
Broodstock survival (%)	58	67	55
ablated	63	63	68
unablated	54	71	41

more fecund and had higher broodstock survival, suggesting that ablation did not bring undesirable stress to the crabs.

Larval quality based on larval stage index (LSI) or percentage success in larval rearing were higher in intact females fed T1 and T3 diets (LSI = 5.3). However, the reverse was true in females fed T2, with LSI = 6; all zoea larvae from ablated females fed this diet attained the megalopa stage. Overall, better results (LSI = 5.0 - 5.2) in terms of success in larval rearing were obtained in this study compared to previous results (LSI = 3.0 - 4.0) with broodstock caught by fishermen from the wild (Millamena 2000). In this study, rematurations were observed in all dietary treatments in both unablated and intact females with no apparent decline in reproductive performance.

Feeding *S. serrata* females on a defined diet in grow-out ponds enabled them to improve the nutrient reserves that are subsequently needed in egg development. The results further suggest a need for an artificial diet as supplement to natural food in order to promote more consistent spawning of good quality eggs and improve consistency of larval production in crab hatcheries.

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