Nauplii Production from Wild, Cultivated, and Mixed Populations of Blue Shrimp, *Penaeus stylirostris*

Roberto Mendoza

ABSTRACT. Due to the low nauplii production of cultivated broodstock, and to reduce the dependence on the wild stock, an experiment was carried out with 400 adult blue shrimp, *Penaeus stylirostris*, from wild and cultivated (F6) populations. Four treatments, each in duplicate, were applied: (1) wild females and males (W-W); (2) wild females and cultivated males (W-C); (3) cultivated females and wild males (C-W); and (4) cultivated females and males (C-C). More than 300 individual spawns were monitored to evaluate the egg and nauplii production per female. Mixed model ANOVA for factorial arrangements ($4 \times 3 \times 2^3$ and 3×2^3) were conducted. The factors considered besides the treatments were: rematuration (number of successive spawnings for a female), ovarian maturity, integrity of the spermatophore attached (complete spermatophore, "wings," or "remnant") and condition of spawning (partial or complete). The introduction of both wild females and males was a successful measure to improve the overall egg and nauplii production. Both mixed populations outperformed the cultivated broodstock, but were inferior to the wild stock (average production of eggs and nauplii: W-W-112,713 and 34,682, respectively; W-C-113,215 and 22,038, respectively; C-W-82,702 and 11,715, respectively; C-C-66,948 and 7,653, respectively). Populations with wild females produce a larger number of eggs, and wild males contribute to higher hatching rates. Other observations indicate the need to select for spawning only those females showing an advanced degree of ovarian maturity, having a complete spermato-

Roberto Mendoza, Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Laboratorio de Maricultura, Apartado Postal F-56 C.P. 66450, San Nicolás de los Garza, N.L., México.

Journal of Applied Aquaculture, Vol. 7(2) 1997

phore attached to the thelycum, and spawning completely. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]*

INTRODUCTION

There are many locations along the Mexican Pacific coast where land and environmental conditions are suitable for aquaculture of marine shrimp, and there is an optimistic outlook for a large-scale, year-round shrimp aquaculture industry. A major drawback in the shrimp industry is the lack of reliable sources of seedstock. This problem can be solved by means of producing a constant and predictable number of postlarvae to supply the farming operations (Johns et al. 1981). This can be achieved only by optimizing a system for penaeid reproduction in captivity.

Although postlarvae can be obtained throughout the year, it has been reported that captive broodstock produce a lower number of nauplii (3×10^4/female) when compared with the wild stock (5×10^4/female), despite the former being raised under controlled conditions (Serralde and Almada 1985).

Due to the need to improve the efficiency of maturation spawning systems, a new approach has been initiated, consisting of combining wild-caught broodstock with captive shrimp as a short-term measure to increase the larva production of cultivated broodstock. The present study was conducted to determine the use of cultured and wild broodstock and the role of some major factors such as the effect of successive spawnings, female maturity stage, integrity of spermatophore (complete or incomplete) when attached to the thelycum of the female, and spawning condition (partial or complete), which may affect egg and nauplii production.

MATERIALS AND METHODS

Experimental Design

Four experimental treatments, each in duplicate, consisting of the combination of 30 blue shrimp adults (1:1 sex ratio) from wild and cultivated (F_6) populations: (1) wild females and males (W-W); (2) wild females and cultured males (W-C); (3) cultured females and wild males (C-W); and (4) cultured females and males (C-C), were cultured to determine their performance in nauplii and egg production. Moreover, four factors were considered in the evaluation of the above mentioned treatments: rematuration (number of successive spawnings for a female), ovarian maturity, integrity

of the spermatophore (complete spermatophore, "wings," or "remnant"), and spawning condition (partial or complete spawning). Raceway units of 22 m^2 were randomly assigned to each treatment. Resulting spawnings of the females from the different treatments were evaluated over a period of six months.

Maturation Experimental Tanks

The maturation-reproduction process was carried out at the facilities of the Center of Scientific and Technological Research of Sonora University in Mexico. Raceways (23 m long × 3 m wide × 0.4 m high) were housed under plastic structures, as those described by Mahler et al. (1974). Water flow allowed a daily turnover of 400%. Neither biological filters nor water recirculation was used.

Spawning Tanks

Fiberglass tanks used for spawning and hatching (50 cm long × 50 cm wide × 49 cm high) were filled to a volume of 100 L. Incoming water flow was maintained at a rate of 210 mL/min, allowing a daily exchange of 300%. Water was drained through a central filter with a screen of 0.1 mm mesh opening. Two airlifts were placed in each tank for total water circulation. Continual flow was maintained only during spawning.

Water Quality

Water was pumped from an ocean well; the surrounding sand acted as a filter medium. Removal of waste products was ensured by maintenance of appropriate flow rates. Temperature and oxygen were monitored daily by YSI oxygen meter (model 54ARC, YSI, Yellow Springs, Ohio[1]) as well as salinity (AO refractometer, Model 10419, Japan); total ammonia was monitored weekly (Ammonia Nitrogen Kit, Model NI-8, HACH, Loveland, Colorado). Water was heated by means of a water heater when needed. Aquaria were cleaned every three days to avoid excessive algal growth. Water for spawning was additionally filtered through a sand filter. The water quality conditions throughout the experimental period were (± S.D.): water temperature 27° ± 2.5°C; salinity 38 ± 1.2 ppt; and the natural photoperiod evolved from 11 light hours at the beginning of the experiment (March) to reach a maximum of 14.5 hours in May-June; finally decreasing to 13 hours at the end of the experiment (August).

1. Use of trade or manufacturer's names does not imply endorsement.

Broodstock History

Wild blue shrimp were collected from the Gulf of California near Puerto Peñasco, Mexico. Shrimp (mean weight 58.8 g) were stocked at a density of 2.3 individuals/m^2 and were fed a maturation diet just after their arrival at the facilities and for one month before the experiment began.

Cultivated broodstock were raised from eggs spawned from the fifth generation and were maintained at a density never exceeding 300 g/m^2 until they reached reproductive size. Mean weight at the beginning of the experiment was 53.4 g (after 13 months of growth). They were stocked at the same density as the wild stock.

Animals were fed a formulated cold-extruded, pelleted diet, having a proximate analysis of 28.4% protein, 3.5% fat, 3.4% fiber, and 4.1% kcal/g. Diet was supplemented with frozen squid, *Loligo* sp., and was supplied at a daily rate of 3% total biomass.

Dead shrimp were removed every morning and examined to determine the cause of death. Individual records were kept according to the sex and experimental treatment.

Maturation and Spawning

All females were marked with numbered metallic tags as described by Rodriguez (1976), to identify them during spawning for the rematuration record. No eyestalk ablation or artificial insemination was performed. Females were examined for ovarian maturation and mated condition. This was accomplished with a hand-net and an underwater flashlight. the translucence of the shrimp's body permitted observations through the dorsal part of the abdomen for the shape and color of the posterior lobe of the ovary. Females with attached spermatophores were introduced into PVC tubes with longitudinal slots. These tubes were left in the raceway units where females were found. When mated animals were no longer available, all the tubes were collected in a bucket and transported to the spawning tanks. Inspections were carried out every third night.

Each female was placed into a spawning tank and left overnight. After spawning, the tank was gently drained, and eggs were washed and concentrated in a device with two series of mesh (one of 330 mm to remove follicular material and one of 110 mm to retain the eggs) similar to those described by AQUACOP (1982a) and Primavera (1983). Eggs were poured into another tank with clean filtered water treated with EDTA (0.01 g/L) and erythromycin phosphate (5 mg/L). Aliquots of 250 mL were taken for counting eggs and nauplii.

At the termination of the experiment, 16 females were selected from each treatment for dissection to determine the gonadosomatic index (GSI).

Statistical Analysis

The experiment was designed as completely randomized and in order to appraise the significant differences in nauplii production and egg production, two factorial arrangements were performed. The factors considered were:

Four populations

wild females and wild males (W − W)
wild females and cultivated males (W − C)
cultivated females and wild males (C − W)
cultivated females and cultivated males (C − C)

Three rematurations (successive spawnings)

S1 = 1st spawning
S2 = 2nd spawning
S3 = 3rd spawning

Two ovarian maturity stages

Low maturity stage (developing ovary)
High maturity stage (fully mature)

Two conditions for the spermatophore

complete
incomplete ("wings" or "remnant")

Two spawning conditions

complete spawning (the whole ovary was spawned)
incomplete spawning (only part of the ovary was spawned)

Originally, four maturity stages were recorded, based on the apparent ovary volume according to the criteria described by King (1948), AQUACOP (1975) and Chamberlain and Lawrence (1981), but to increase the precision of the ANOVA, the four levels of this factor were compressed into two: low maturity stage (LMS) and high maturity stage (HMS).

In the case of the spermatophore integrity, data were recorded for complete spermatophore; this designation refers to the compound spermatophore as described by Cardenas (1952) and Perez-Farfante (1975); "wings" refer to the wing-like processes that are part of the spermatophore, and "remnant" refers to the part of spermatic masses. The three levels of this factor were compressed into two: complete spermatophore (CS) and incomplete spermatophore (IS).

Factorial Arrangements

To evaluate nauplii production, ANOVA for a $4 \times 3 \times 2^3$ factorial arrangement (corresponding to four populations, three rematurations, two ovarian maturity stages, two conditions for the spermatophore and two spawning conditions) was conducted. All the above-mentioned factors were considered. The ANOVA was performed as a mixed model. Population (which includes the experimental treatments) was considered fixed, and the rest of the factors were considered random. Because it is not always possible to find a suitable error term to test the main effects and interactions, it was necessary to pool some of the mean squares by Satterthwaite's procedure to obtain an F-like test criterion ("quasi-F ratio") that distributes approximately as F (Montgomery 1976; Steel and Torrie 1980).

For egg production, a mixed model ANOVA was carried out for a 3×2^3 factorial arrangement. The factors considered were: rematuration, maturity stage, integrity of the spawning, and source of the females (wild or cultivated). When significantly different interactions were found, the average responses were analyzed for the levels of each factor.

The new Duncan's Multiple Range test was used to detect and isolate differences among the levels of the different factors. Also differences between values were determined by using the two-tailed Student's test ($P = 0.05$)

RESULTS AND DISCUSSION

During the 180-day period, a total of 304 spawnings were noted, with the production of 26,781,049 eggs and 6,138,380 nauplii. Results indicate that wild females produced a larger ($P < 0.05$) number of eggs (18,382,720) when compared with cultured females (8,398,329), which may be related to the higher individual values of the gonadosomatic index for wild females (up to 7.88), in spite of the fact that no significant differences ($P > 0.05$) were found between mean $\pm$ S.D gonadosomatic indices for wild (4.47 ± 1.63) and cultivated spawners (3.57 ± 1.47).

The increase in egg production was reflected in the average nauplii production (Table 1). Populations with wild females gave better results when compared with populations where females from the captive broodstock were present. On the other hand, wild males contributed to higher hatching rates than those for cultivated males with females of the same source (W – W had a 32% hatching rate in comparison with W – C = 20%, while C – W had 14% in comparison with C – C = 10%), ($P < 0.05$).

Average nauplii production was increased when females with larger ovaries presented a complete spermatophore attached ($P < 0.05$) and when they spawned completely ($P < 0.05$), regardless of the spawning rank. Only during the first spawn did the production increase, even when the spermatophore was incomplete.

The number of days elapsed between successive spawnings and the percentage of spawns associated with high maturity stages decreased with rematurations, but this did not affect the spawning condition (complete or incomplete). On the contrary, a rise in the average egg production was observed from the first to the third spawn.

The main causes of mortality were molting (51%), handling of shrimp during counting and sexing (21%), shrimp jumping out of the aquaria (20%), and disease (8%). Boat-shaped conidiospores were found on the surface of lesions, which are characteristic of *Fusarium* sp. (Sindermann 1977). The overall survival of females (51.9%) was lower than that of males (58%).

Results comparing average egg and nauplii production revealed that both mixed populations outperformed the cultivated broodstock. The higher nauplii production of the population containing wild individuals

TABLE 1. Mean ± S.D. production of eggs and nauplii per treatment. Combined populations were: wild females and males (W – W); wild females and cultured males (W – C); cultured females and wild males (C – W) and cultured females and males (C – C). Within a column, values followed by different letters were significantly different ($P < 0.05$).

Treatments	Eggs	Nauplii	Hatching rate	No. spawnings
W – W	112,713 ± 65,131a	34,682 ± 38,697a	32.40 ± 33.10a	78
W – C	113,215 ± 70,832a	22,038 ± 34,539ab	20.12 ± 26.65a	84
C – W	82,702 ± 56,107b	11,715 ± 20,654c	20.77 ± 30.57a	46
C – C	66,948 ± 50,199b	7,653 ± 18,123c	11.64 ± 19.91b	68

was a consequence of the higher frequency of spawns associated with advanced stages of ovarian development for wild females. One possible explanation may be the larger size of wild females, which agrees with the findings of Menasveta et al. (1994), who observed that larger females underwent more advanced stages of maturation and spawned with greater success than did small prawns. In relation to the higher gonadosomatic index reached by wild females, it should be noted that a similar observation was reported by Lawrence et al. (1979). Spawning of cultivated females with a narrower ovary width than wild spawners has also been noted in the case of other species (Emmerson 1980). Furthermore, the fact that wild males were also slightly bigger than cultivated ones may have contributed to higher hatching rates. According to Pratoomchat et al. (1993), larger shrimp produce significantly larger spermatophores and quantities of sperm than small prawns. These results agree with the finding of other authors who have pointed out the differences in fecundity between wild and cultivated spawners (Moore et al. 1974; Magarelli 1981; Primavera 1982).

The lower quality of eggs obtained from the captive broodstock has been associated either with nutritional deficiencies (AQUACOP 1982b) or with living under artificial conditions (Lumare 1981; Magarelli 1981). Bray and Lawrence (1992) stated that potential breeders in captivity are subjected to unnatural noise levels, close confinement, frequent handling, different substrate, unnatural lighting and photoperiod, unavailable live food, and often marked differences in water quality; all these conditions, in our experience, adversely affect spawning.

Spawning frequency was lower in those populations where cultivated individuals were present. Within cultivated populations, C–W matings showed a higher overall production, although presenting a lower spawning frequency than C–C broodstock.

Higher hatching rates were observed during the first spawning. The main cause seems to be the higher percentage of spawns associated with high maturity stages (74%), in contrast with the second and third spawnings (66 % and 56 %, respectively), where females have mated when their ovaries were still in developing stages. This tendency of ovaries to show a lesser degree of enlargement with successive spawns has been explained as exhaustion of females (Lumare 1979).

The average number of nauplii/female obtained during the present study was low, in comparison to that reported by others. (Brown et al. 1980; AQUACOP 1982a). Different maturation conditions provided in those studies, such as practice of eyestalk ablation, the size of the animals, and different water quality conditions may be possible explanations for the differences.

Results of this study suggest that the combination of wild individuals of blue shrimp with cultivated ones is a successful short-term measure to improve the nauplii production regularly obtained with captive broodstock. Furthermore, to obtain good quality spawnings, it is necessary to select only those females showing an advanced degree of ovarian maturity, having a complete spermatophore attached to the thelycum, and spawning completely.

ACKNOWLEDGMENTS

This study was part of a research program of the maturation section of the C.I.C.T.U.S. Shrimp Aquaculture Area. The author thanks the night technical staff of the U.E.P. for their help in the practical work of this study, to the researchers of this center for their assistance in the preparation of this manuscript, and to CONACyT for sponsoring part of this research.

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