Studies on the Artificial Insemination and Fertilization of Grass Shrimp, *Penaeus monodon*

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The culture of grass shrimp, *Penaeus monodon* has become a fast-growing enterprise in Taiwan since formulated shrimp feed was successfully developed in 1978. In 1983, the total postlarval production for stocking reached 600 million at the price of 12.5 U.S. cents each. This high price of the postlarvae resulted from (1) limited availability of wild gravid females, (2) undesirable spawnings obtained by using the method of eyestalk ablation, manifested by a low average hatching rate of 20%, and (3) high demand from grow-out farms. The eyestalk ablated females induced to spawn were often found unmated which partly explained the poor spawnings and low hatching rates. Consequently, re-use of ablated females was not practised by farmers in the past.

The present paper describes the results of artificial insemination and fertilization of wild or pond-reared females whose gonadal development was induced by eyestalk ablation. The hatching rates from unmated soft-thelycum females implanted with two spermatophores are 84.7% and 43.7% while those implanted with only one spermatophore, 74.1% and 16.8%, for the first and subsequent spawning, respectively. These results positively confirm that the unmated condition of ablated females is the main reason for low hatching. Through artificial insemination, the spawning and hatching can be improved and ablated females can be reutilized. For unmated hard-thelycum females, artificial fertilization was done by releasing spermatozoa into the spawning tank right before spawning. Out of 15 attempts, three were successful with hatching rates of 63.1, 52.3, and 49.9%.

Induced maturation of pond-reared shrimps was attempted by manipulation of temperature and salinity. Under constant temperature of 22±2°C, salinities ranging between 25 and 37 ppt were experimented. The best results with 67% success were obtained at salinities of 30 and 35 ppt. Continued efforts will be made to improve spawning performance through the technique of artificial insemination under controlled conditions.

Factors Affecting Maturation and Spawning of *Penaeus esculentus* in the Laboratory

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Adult tiger prawns *Penaeus esculentus* were held in laboratory tanks under varying conditions of tank size, density, temperature and photoperiod for assessment of ovarian maturation and spawning. Both eyestalk ablated and intact females were studied. Maturation and spawning of intact females was favored by conditions of warm temperature (26°C) and long days (14.5 hr), whereas ovary maturation did not occur at lower temperature (20°C) and short days (12 hr). Tank size was a critical factor with intact females as maturation and spawning required a large tank (4 m^2). Spawning did not occur in small tanks (1 m^2) despite ideal temperature and photoperiod conditions. Unilaterally ablated females matured and spawned under both short day-cold temperature conditions and in small tanks, but the success rate was greater under long day-warm temperature conditions in large tanks. Intact females required 40-60 days before onset of ovary maturation, whereas ablated females showed maturation to ovary stage III approximately 20 days after ablation. Mating success was severely limited under small tank conditions but occurred normally in the large tanks.

Induction to Ovary Maturation by Ablation in the Pink Shrimp *Penaeus notialis*

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A partial unilateral ablation was carried out on immature females of the pink shrimp *Penaeus notialis*. They were maintained in 1,600 ℓ asbestos-cement tanks together with apparently mature males, not submitted to treatment, at a ratio of 2 females: 1 male. A quick development of the ovary was attained, which did not present significant differences in average diameter of the ovocytes in the anterior, median, and posterior lobes, and with similar histological characteristics to those described for naturally mature females. Viable spawnings were obtained three days after the treatment and onwards. The larvae obtained showed normal activity and development.

Observations on the Nauplii Production from Wild, Cultivated and Mixed Populations of the Blue Shrimp (*Penaeus stylirostris*)

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Due to low nauplii production from cultivated broodstock and to minimize dependence on wild stock, an experiment was run in which four treatments, consisting of combina-

tions of 400 adult blue shrimp (*Penaeus stylirostris*) from wild and cultivated (F_6) populations, were applied (wild females and males, wild females and cultivated males, cultivated females and wild males, and cultivated females and males). Females were inspected every third day. Those observed with spermatophores were captured and transferred to individual 100-ℓ spawning tanks. Water was treated with EDTA and erythromycin phosphate. More than 300 individual spawns were evaluated within a 180-day period. To evaluate the nauplii production per female, an analysis of variance for a factorial arrangement ($4^3 \times 2$) was conducted. The factors considered were: the abovementioned treatments, different ovarian maturation stages, adhesion of the spermatophore, and kind of spawning (complete or partial). The mixed populations had higher nauplii production than the cultivated broodstock. All the females were tagged around an eyestalk and examined for rematuration. Up to six rematurations per female were registered as well as a minimum of four days between successive spawnings for the same female. The effect of rematuration on the quantity of nauplii is discussed. Gonadosomatic index for wild and cultivated females is compared. Selective criteria for spawners are given.

Nutritional Value of Marine Yeast Fed to Larvae of *Penaeus monodon* in Combination with Algae

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Saccharomyces cerevasiae and *Rhodotorula aurantica*, two marine yeast species, were fed to *Penaeus monodon* larvae (N_6 to M_1) singly and in combination with *Tetraselmis* sp. and *Chaetoceros calcitrans* in varying proportions. Larvae fed combination diets gave survival rates comparable to or higher than those fed algae or yeast alone. Chemical analyses show that the yeasts have low fat, moderate protein and high carbohydrate content. They also contain essential amino acids but are different in the fatty acids found to be essential for prawns. When used in combination with algae, the nutritional value of the yeasts seemed to have been improved.

The use of marine yeasts in larval rearing could reduce economic and technological inputs in the production of natural foods for larval rearing. They are cheaper and easier to mass produce. They can be grown to very high densities using cheap carbon sources like molasses, brown sugar and coconut water with added nutrients in relatively shorter periods of time.

The Growth of a Bialgal Culture and its Use as Food for Shrimp Larvae

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The cultivation of the microalga *Tetraselmis chuii* with the protection of the extracellular products of *Chlorella kessleri*, grown in a bialgal culture, allows its development in outdoor tanks without special conditions of sterilization or aeration. Fish meal and agricultural inorganic compounds are used as fertilizers. The growth of the mixed species is analyzed comparing it with monoalgal cultures. The best fit of growth data to a logistic curve is performed and the whole curve is compared using a covariance analysis. The stratification of *T. chuii* in the tank favors its harvest at high concentration. A bialgal culture (based on *T. chuii* at 50 cells/mm^3) as food for the larvae of the shrimps *Penaeus notialis* and *P. schmitti*, together with hard boiled egg yolk and rotifers, achieves good development and survival.

The Integrated Use of *Artemia* in Shrimp Farming

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The use of freshly hatched *Artemia* nauplii in penaeid hatcheries is a common practice, although a broader application of *Artemia* in shrimp farming is gaining more and more interest. In this regard, an integrated use of *Artemia* in shrimp culture is presented in this paper.

Artemia booster in combination with Fleischmann yeast has been proven to be a suitable algal substitute and the early feeding of decapsulated *Artemia* cysts at protozoea I to II stages has been shown to improve larval growth. Freshly hatched *Artemia* nauplii may be introduced at protozoea II to III and the use of enriched nauplii from mysis stage on clearly improves postlarval production. Enriched nauplii, pre-adult and adult *Artemia* can be successfully used in a nursery phase in order to improve weaning success and performance in grow-out ponds. Furthermore, the use of adult *Artemia* in broodstock feeding has been shown to be effective for inducing maturation.

All *Artemia* products mentioned can be purchased from commercial dealers but can be produced as well on the spot in