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Improved Maturation of Wild and Pond-reared Black Tiger Shrimp *Penaeus monodon* (Fabricius) using different Combinations of Live and Wet Feeds

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Abstract : The impact of various combinations of live feeds and frozen feeds such as polychaete, squid meat, crab meat and beef liver was tested on the maturity and spawning of the shrimp *Penaeus monodon.* Females with weight ranges 86g to 96g were selected for the present experimentation. The shrimps were fed with five different combinations at the rate of 15% of their body weight. Feed combinations selected for current study was crab and polychaete, oyster and polychaete, squid and clam, beef liver and squid, and oyster and beef liver. We applied different ablated methods to evaluate the reproductive ability of wild and pond reared *Penaeus monodon* The great reproductive performance was achieved when shrimps fed with Feed-I, the reproductive performance based on fecundity was identified by the number of eggs in gravid females. Future research efforts focused on the artificial diets which contain both live and frozen feed combinations will be essential for the improved egg quality in tank-reared *Penaeus monodon*. Thereby increasing the consistency and reliability of maturation diets for better quality nauplii production.

Key Words: *Penaeus momodon,* Spawning, Fecundity, Hatching, nauplii, Survival rate

Introduction

In addition to eyestalk ablation, various naturally occurring and formulated feeds are proved fertile in inducing maturation in pond reared and naturally collected penaeid shrimps. Fish meal, Vitamin-C, and fish oil play important role in growth and reproduction in aquatic animals (Jia-Yuan Xu, *et al.,* 2010). In general the fresh or frozen diets currently used for maturation of shrimps under captivity have (1) rich animal protein (approximately 60% on dry weight basis) with amino acid profiles similar to that of shrimps (e.g. shrimp and some bivalves such as Tapes sp.) (2) High cholesterol and (3) Phospholipids, with Phosphatidylcholine (Lecithin) fraction which is probably of major importance. Other than these generalities, little is known of maturation diets. Nevertheless, the inhibition of ovarian development under captive conditions was overcome by eye stalk ablation (Beard and Wickin, 1980). Unilateral eye stalk ablation is simple techniques for seed production (Villaluz *et al*., 1972).

Food is an important factor for sexual maturation (Browday, 1992) and male reproductive performance (Meunpol *et al*., 2005) Many advances were made in the development of technology for maturation and reproduction of penaeid shrimp (Bray and Lawrence, 1992 and Browday, 1992). The present study was undertaken to demonstrate the larval survival of ablated pond – reared *P. monodon* by using five different practical diets.

Materials and methods

For studies on the impact of fresh feeds on fecundity & hatching rates, the brooders , raised in shrimp farms were transferred to 10 ton capacity epoxy resin coated rectangular RCC tanks of 52.51 m for further development as spawners. The initial weight of females and males ranged from 86.18 to 96.52 g and from 50.61 to 65.21 g respectively. The shrimps were fed with five different feeds combinations as crab and polychaete, oyster and polychaete, squid and clam, beef liver and squid, and oyster and beef liver. Shrimp were fed at the rate of 15% of their body weight. It took about 60 days for the conversion of pre-vitellogenic female into the vitellogenic stage. The developed spawners were transferred to a hatchery to assess the efficiency of spawners in terms of spawning, hatching and survival of larvae.

The gravid females were transferred to hatchery, where they were gradually acclimatized to the hatchery conditions and were disinfected with 10 ppm of formaldehyde and 50 ppm of Treflon (Trifluralin) respectively for 20 minutes. Spawners were fed at the rate of 15-20% of their bio-mass at regular interval of time both during the day and night. Water temperature ranged from 28-31C and sea water salinity 29-32.5 ppt in the hatchery and also in the laboratory. Prior to initiation of the trail, each spawner was weighed and the weight was recorded. Spawners were stocked at the rate of 2-3 m^2 in female to male ratio of 2:1. Females were checked for the presence of spermatophores in the thelycum. Unilateral and bilateral eyestalk ablation was carried out in the early morning hours during the intermoult stage by cutting the eyestalk with sterilized scissors. After eyestalk ablation and a period of five to seven days, females were checked by with under water torchlight (sourcing light) for ovarian maturation by external observation. Based on the relative size of the ovary gravid females in maturation stage IV were transferred to round fibre tanks having 0.5 ton of filtered sea water of 30 ppt for spawning. After spawning, the

water was drained using fine meshed net (100 µ mesh) to retain the eggs. The spawned eggs were washed with treated seawater containing Treflon as anti-fungal reagent, concentration ranging from 0.02 to 0.05 ppm. After 2 to 3 washings, the eggs were stirred and three one ml aliquots were drawn from the beaker to count the number of eggs. Then the eggs were transferred to 500 litre capacity black paint coated hatching tanks. After 40-45 minutes of spawning and upon the appearance of hatching envelop, the number of nauplii in 50 ml aliquots drawn from the hatching tanks was enumerated. The hatching rate was calculated by the following formula.

$$\text{Hatching rate (\%)} = \frac{\text{Total nauplii}}{\text{Total eggs spawned}} \times 100$$

Morbid larvae and Dead larvae were removed from larval rearing tanks by siphoning. A water temperature of 29-31C, pH 7.8, and salinity of 29-32.5 ppt were maintained at the optimum levels throughout out the study period. For studies on larval diet and larval development, the treated seawater as said above was used in the laboratory and hatchery. The larval rearing tanks (LRT) have capacities of 14-16 tons, which are parabolic in their structure and design.

The larvae were maintained in 1.2 m 7.5 m 1.8 m size tanks. The water level in each tank was maintained at 16 tones. Tanks with a water holding capacity of 14 tonnes were used for rearing the post larvae. A haemocytometer was used for algal cell counting in the medium. A salinity refractrometer was used for regular checking of the water salinity. Algal culture was done in 250 ml, 500 ml, 1000 ml and 2000 ml conical flasks (Borosil glass) and 20 litre carboys were used for indoor algal culture. 100 litres plastic cans, 1000 litre to 1200 litre FRP tanks, and 4 tonnes capacity rectangular concrete outdoor tanks were used for outdoor algal culture. Round, clylindro-conical fibre glass tanks of 500 litres capacity were used for artemia culture.

The various prophylactic measures taken with treatment groups are presented in Table I. In all the treatments, the water quality parameters such as temperature and salinity were in optimal range of 29-31C, 28-32 ppt respectively.

Results

An important aspect of the present study is on the influence of feeding condition on the growth and maturation of shrimps under captivity. This study is aimed in filling up the gap in aquaculture industry. Considerable variations were noticed when the shrimps were fed with combined fresh feeds. The feeds have remarkable influence on the growth body weight, and also on fecundity rates.

The maximum Average daily growth (ADG) of 0.401 g was recorded when the female shrimps were fed with crab and polychaete. In males the highest average daily growth (ADG) of 0.301 g, was observed when the shrimps were fed with crab and polychaete, while the lowest average daily growth (ADG) of 0.257 g in female shrimps was recorded when the shrimps were fed with oyster and beef liver. The lowest average daily growth (ADG) of 0.183 g was recorded in male shrimps fed with oyster and beef liver (Fig.1 & 2).

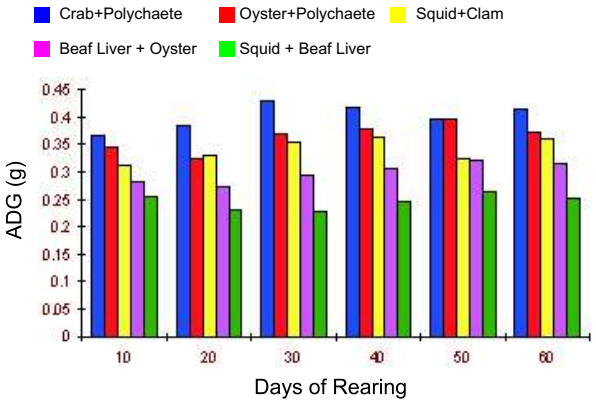

Fig. 1. ADG of Female shrimps (P. monodon) fed with different feed combinations

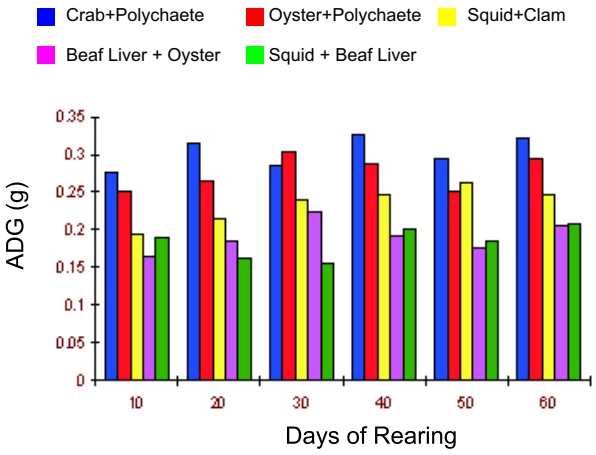

Fig. 2. ADG of Male shrimps (P. Monodon) fed with different feed combinations

A notable difference in body weight gain was recorded among the shrimps fed with different combinations of feed. The maximum weight gain of 24.06 g was noticed in females fed with crab and polychaete in 60 days of time, whereas in males the weight gain obtained was 18.12 g in the same duration of feeding condition and with the same feed. The results with respect to the average daily growth (ADG) and net body weight are summarized as follows.

Females

Average Daily Growth (ADG): Crab and polychaete, 0.401 g; oyster and polychaete, 0.364 g; squid & clam, 0.340 g: beef liver and squid, 0.298 g; oyster and beef

liver 0.257 g. (Fig.1)

Net weight gain in 60 days: Crab & polychaete, 24.06 g; oyster and polychaete, 21.84 g; squid & clam, 20.40 g; beef liver and squid, 17.88 g; beef liver & oyster, 15.42 g; (Fig.3).

ADG of Males:

Crab and polychaete, 0.302 > oyster and polychaete 0.273 > squid and clam 0.234 > beef liver and squid 0.191 g > oyster and beef liver, 0.183 g. (Fig. 2).

Net weight gain: Crab and polychaete 18.12 > oyster and polychaete 16.38 > squid and clam 14.04 > beef liver and squid 11.46 g > oyster and beef liver 10.98 g. (Fig. 4)

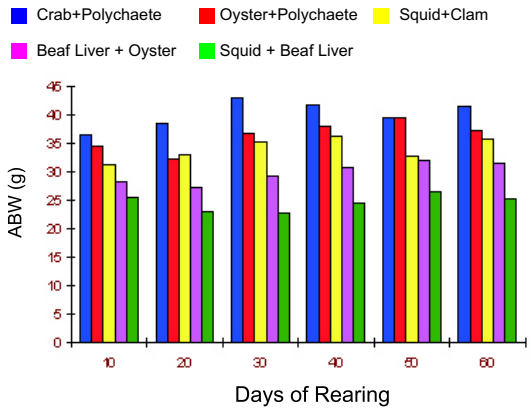

Fig. 3. ABW of Female shrimps (P. monodon) fed with different feed combinations

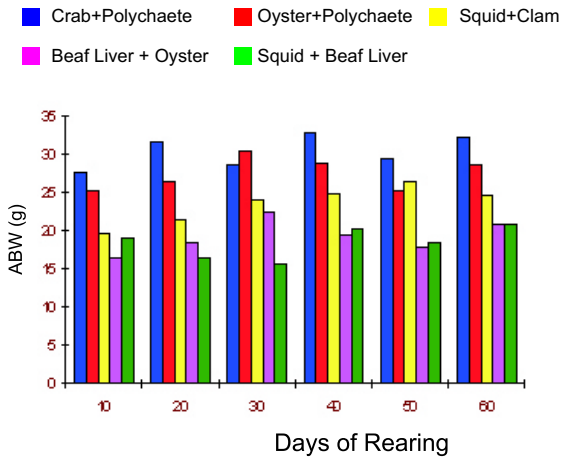

Fig.4. ABW of Male shrimps (P. Monodon) fed with different feed combinations

Maturation

Ovarian maturation in shrimps fed with different combinations of feeds was found to vary significantly between the treatments. The minimum elapsed period recorded among wild unilaterally ablated was 6-9 days in shrimp fed with crab and polychaete followed by 5-10 days in shrimp fed with oyster and polychaete and the maximum of 10-16 days was recorded in the case of shrimp fed with oyster and beef liver (Table 1)

The minimum elapsed period recorded among captive unilateral ablated was 9-15 days in shrimp fed with crab and polychaete, and the maximum of 19-27 days was recorded in case of shrimp fed with oyster and beef liver. The minimum elapsed period in recorded among wild bilateral was 10-13 days in shrimp fed with crab and polychaete, and the maximum of 20-24 days was recorded in case of shrimp fed with oyster and beef liver (Table 1)

Table: 1. Efficiency of different fresh feed combinations on maturation of shrimps (elapsed time) from first stage to spawning (days).

Feed combinations	Wild unilaterally ablated	Captive unilaterally ablated	Wild Bilaterally ablated
Crab and polychaete	6 to 9	9 to 15	10 to 13
Oyster and polychaete	5 to 10	11 to 16	12 to 18
Squid and clam	6 to 13	13 to 20	15 to 20
Beef liver and squid	8 to 15	16 to 24	18 to 22
Oyster and beef liver	10 to 16	19 to 27	20 to 24

Wild unilaterally ablated

Experiments on eyestalk ablation were conducted on shrimps collected from the wild (from the Sea) and the following observation ware made. Crab and polychaete 6-9 days > oyster and polychaete 5-10 days > squid and clam 6-13 days > beef liver and squid 8-15 days > oyster and beef liver 10-16 days (Table 1).

Captive unilateral ablated:

Eyestalk ablation was conducted on shrimps initially reared in aquaculture ponds and the results are like this. Crab and polychaete 9-15 days > oyster and polychaete 11-16 days > squid and clam 13-20 days > beef liver and squid 16-24 days > oyster and beef liver 19-27 days (Table 1).

Wild bilateral ablated:

Experiments on eyestalk ablation were conducted on shrimps collected from the wild (from the Sea) and following observation were made. Crab and polychaete 10-13 days > oyster and polychaete 12-18 days > squid and clam 15-20 days > beef liver and squid 18-22 days > oyster and beef liver 20-24 days (Table 1).

Spawning rate:

A comparison is made on the number of eggs laid per each brood in the shrimps raised under captivity and also shrimps caught in the sea. For this care is taken to include the same weight groups of animals and it is clearly evident that the number of eggs laid by the shrimps caught in the sea are significantly higher when compared to those that were raised under captivity Shrimp fed with crab ad polychaete and oyster and polychaete showed interesting results of higher spawning.

Hatching rate:

Hatching rate was more or less the same for successive spawning in ablated females. This could be related to batch availability and variability of sperm in the thelycum of the female. Hatching rate was more in shrimps collected from the Sea. Among wild ones the maximum hatching rate of 90.56 was recorded in unilateral ablated wild shrimp fed with crab and polychaete and the minimum of 69.85 in the unilateral ablated wild shrimp fed with oyster beef liver. The maximum number of nauplii hatched was 71.05 in the unilateral ablated captive shrimp fed with crab and polychaete and minimum of 32.25 captive shrimp fed with oyster and beef liver. In the wild bilateral ablated shrimps fed with crab and polycheate maximum 72.75 and minimum of 27.45 shrimp fed with beef liver and oyster respectively (Table 2).

Discussion:

Table: 2. Percentage of hatching rate in wild and captive spawners

Feed combinations	Wild unilaterally ablated	Captive unilaterally ablated	Wild bilaterally ablated
Crab and polychaete	90.46 ± 1.34	71.05 ± 1.72	72.75 ± 1.20
Oyster and polychaete	84.25 ± 2.44	53.13 ± 1.05	65.20 ± 2.83
Squid and clam	72.66 ± 1.95	42.83 ± 1.42	51.99 ± 1.56
Beef liver and squid	78.65 ± 1.93	40.88 ± 1.27	45.82 ± 1.68
Oyster and beef liver	69.85 ± 1.57	32.25 ± 1.16	27.45 ± 1.17

*Mean values standard deviation

As far as hatchery production of shrimp seed is concerned, continuous production of high quality nauplii has certainly remained an elusive goal. In this backdrop, the greater and better ovarian maturation and spawning success in captive spawners developed by feeding crab and polychaete was better, this argues in general with the findings of (Brown *et al*., 1979, Primavera *et al.*, 1979, Beard and Wickins, 1980, Chamberlain & Lawrence 1981, Menasveta *et al.*, 1994 and Sangpradub *et al*., 1994). In order to estimate the ecosystem support area that is needed to supply hatcheries with shrimp spawners, the supporting ecosystem or habitat type must first be assigned. Present studies will argue that the mangrove ecosystem is the critical habitat for *Penaeus monodon* in Andhra Pradesh, India.

In present studies argue that the mangrove ecosystem is crucial prerequisition for the recruitment success of many penaeid shrimp species, particularly P.monodon. In the state of Andhra Pradesh, the Godavari river delta should be a key nursery ground, since this delta alone harbours 56% of the total mangrove cover in the state. This is also supported by the fact that the coastal areas off the Godavari delta constitute the main trawling grounds for both fin and shell fish in Andhra Pradesh.

Numerous investigations made on shrimp reproduction pointed out that various factors as photoperiod, light intensity, physico - chemical parameters of waters and color of maturation tanks play a vital role (Chamberlain and Lawrence, 1981 and Wyban *et al*., 1987) and eyestalk ablation (Chamberlain and Lawrence, 1981) was reported to be of much help in inducing maturation. When all the requirements are provided, eyestalk ablation is of immense use in inducing gonadal maturation. In the present study, brood stock developed from wild grown shrimps was converted as spawners making use of various feed combinations. Their reproductive performance was tested in a commercial hatchery. Detailed comparison is made with naturally caught shrimps with respect to survival rate, maturation, spawning rate and hatching rate to drive home the advantage of developing brood stock and spawners from farm grown ones due to the inherent merits.

In the feeding experiments, importance has been given to live polychaetes in the present study towards maturation and spawning as suggested by (Brown *et al*., 1979 and Middleditch *et al*., 1979), Next to polychaetes, squid was found to be the best single - food diet, as it produced higher levels (although not significantly higher in every case) of growth, molting, maturation and spawning than the other single food diets, the growth promoting characteristics of squid have earlier been reported (Deshimaru & Shigeno, 1972 and Fenucci et al., 1980). The high maturation performance may be related to the high percentage (98%) of sterol in the form of cholesterol (Lytle and Lytle, 1989) have pointed out that crustaceans such as Artemia and Mactra chinensis (Teshima *et al*., 1988) are considered as good diets with essential feed ingredients for maturation of captive shrimp. Nevertheless, it would appear that mussels are a much more productive in inducing maturation than clams. (Brown *et al*., 1979) found that Penaeus setiferus accepted, mussel more readily than oysters (Crassotrea sp.). suggested that food organisms for maturation should have ripe gonads for ingestion by brood stock and spawner. This would agree well with the present study where the various food items were not reproductively active when they were offered as food and therefore, were less effective than polychaetes which were reproductively active and rich in C 20: C 20 6, C 20:5 3 and C 22:6 3 compounds. Combined diets were proved to be more effective than a single diet.

The relationship between reproduction and growth is dependent up on the division of energy resources. Due to the division of energy towards reproduction a gradual decline in weight of females occurred.

Presently shrimp fed with crab and polychaete showed higher growth rates when compared to others. The next highest growth rate was recorded in shrimps fed with crab and polychaete and oyster and polychaete. The same trend was found in males also, that maximum growth rate in shrimps fed with crab and polychaete and in shrimps fed with oyster and beef liver. Santiago (1977) reported that P. monodon gained an ABW of 16 g. after four months of pond culture. Eyestalk ablation induces maturation in shrimps, whether they are caught in the sea or they are reared in the pond. Elapsed time from eyestalk ablation up to first maturation will be less initially compared to subsequent maturation; more elapsed time also leads to failure of hatching because of low fertilization. (Beard and Wickins, 1982,) found that 22 days of elapsed time in pond-reared females, but with less fertilization rate. Presently, highest elapsed time was recorded in captive animals fed with oyster and cow liver which showed the lowest percentage of hatching. The lowest elapsed time was recorded in both wild and captive spawners fed with crab and polychaete which showed higher hatching rates. Unilateral ablated shrimps showed better hatching rates with low elapsed time compared to bilateral ablated captive shrimps. (Emmerson and Andrews,1983; Hiller, 1984) recorded a higher spawning of 2,57,650 to 5,50,330 eggs per spawn from wild ablated *P. monodon* and 60,000 to 7,47,500 eggs from non-ablated females. The wide range in egg numbers obtained could be due to variations in weight of females (50 - 200g) and inclusion of eggs in counts from both partial and complete spawning (Alikunhi *et al*., 1975; Muthu & Laxminarayana, 1977). However, (Sangpradub *et al*., 1994) found that the average number of eggs per spawner fed with a fresh diet was 1,47,000 contrarily to shrimps with an average mean weight of 136.5 gr. fed with a fresh and pelleted diet yielded 1,39,000 eggs. (Menasveta *et al*., 1994) stated that the total egg production ranged from all 9, 11,460 in smaller (86.68.20 g) to 5, 73,086 in larger ones (135.715.989) reared in the pond.

The result obtained in the present findings indicated highest spawning rate of 4, 45,440 per shrimp in wild unilateral shrimps fed with crab and polychaete. The next highest egg production of 3, 56,487 per spawner was obtained in wild bilateral ablated shrimps fed with the same feed combination. Lowest egg production of 1, 52,945.6 was recorded in shrimp fed with oyster and beef liver of captive unilateral. In pond reared shrimps fecundity and hatching rate were 2, 04,000 eggs. In the present study the hatching rate was found to be low in captive spawners compared to wild ones. The differences in hatching rates may be due to nutrition, sex ratio, water depth and other bio-physical factors. It is also important to maintain certain male: female ration in the maturation tanks.

The highest rate, 90% hatching was recorded in wild unilateral eyes talk ablated shrimps fed with crab and polychaete and lowest hatching rate, 69% was obtained in wild unilateral ablated shrimps fed with oyster and beef liver. The hatching rate in captive raised spawners, 71% as higher and unilateral ablated shrimps fed with crab and polychaete, and minimum lowest of 32% was obtained in shrimps fed with oyster and beef liver. Hatching rate was more or less similar for successive spawning of wild and captive shrimps. Effective fertilization is possible only with males, weighing > 40 g showing signs of full maturity. (Primavera *et al*., 1979, Beard and Wickins, 1980 and Alfaro, 1993), recorded less hatching rate due to insufficient quantity of sperms. Captive and wild shrimp males weighing > 60 g were used in the treatment groups and there were good spawning rates from captive reared spawners as of wild ones. However the hatching rate was low in captive spawner and the reason might be long elapsed time, poor sperm quality and over stocking during spawner development. These should be overcome through further study. In the present work combination of crab and polychaete, and oyster and polyhchaete showed better result in terms of lower elapsed time, high rate of spawning and hatching than other feeds. Improvement of quality of water used in maturation tanks, spawning and hatching tanks through UV radiation found to reduce the levels of specific pathogenic micro organisms in the incoming sea water may be of help in the overall improvement of the reproductive performance. Efforts should also be made to investigate the male shrimps, which will be of help in enhancing the fertilization and hatching rates. Poor water parameters also affect reproductive quality (Perez-Velazquez et al., 2001). Nevertheless, the water quality for all treatments was considered ideal for the species (Peixoto et al., 2005), with temperature between 24.5 °C to 29 °C and salinity of 33 to 35 ppt. Thus, food probably was important factor for aggrieved the spermatophore degeneration reported in the results of the present study.

Conclusion

Our present findings gave satisfactory results on ovarian maturation and spawning in captive spawners, among five different feed combinations crab and polychaete combinations was better which elicit a greater success in the captive and wild caught spawners , further research should continue to establish other good combinations of feeds which will helpful for the production of high quality of nauplii, as hatchery production of shrimp seed is concerned, this will definitely fulfil the gaps in aquaculture.

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