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Procedure for Maturation and Spawning of Imported shrimp *Litopenaeus vannamei* in Commercial Hatchery, South East Coast of India

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Abstract

The reproductive performance of the broodstock was assessed at each stage of the maturation and spawning process. Measures of maturation rate, spawning rate, fecundity, hatch rate and nauplii production rate were obtained. The reproductive performance was found for the imported Specific Pathogen Free (SPF) broodstock from Shrimp Improvement system (SIS), Florida, USA. The no.of spawning, eggs quantity, fertility, hatching rate and nauplii production were lowest in 1st to 3rd spawning when females were 7 months old and increased from 4th spawning rate onwards. As the size of females increased from 30 g to 52 g the mean number of eggs per spawning increased from 150,000 to 442,000. Although female body weight was increasingly larger through the study period and the larger females would be expected to produce more eggs per spawning and hatching. The hatching rate was increased from 3rd spawning onwards. The nauplii production rate (NPR), which is a function of egg production and hatching rate, showed a significant increasing from 48% to 96%. In 10th spawning all the activities were increased. This information can be used to select broodstock for hatchery production.

Keywords: Maturation; Spawning; Fecundity; Hatching; Nauplii; *L. vannamei*

Introduction

The shrimps of the family *penaeidae* are known around the world as valuable resources for aquaculture, but the majority of research and development efforts have been directed to few species (e.g., *Litopenaeus vannamei* and *Penaeus monodon*) that dominate world production [1]. In the last decade, farming of the pacific white shrimp *Litopenaeus vannamei*, of which fast growing and disease resistant strains have been developed by selective breeding programs, has been expanding throughout the world, especially in the far-eastern countries such as Thailand, Vietnam, Indonesia, China and India. This species can be readily reproduced in captivity has wide tolerance to environmental para metres, better utilizes low-protein containing diets and grows fast compared to other penaeid shrimp species [2].

Worldwide commercial maturation of female penaeids relies almost exclusively on the technique of unilateral eye stalk ablation [3,4] the technique give predictable peaks of maturation and spawning, but many associated problems have been reported like deterioration in spawn quality and quantity over time [5-7] and conflicting results on spawn size, hatch success and other variables [3].

The control of ovarian maturation and spawning is a major problem in the development of commercial aquaculture of penaeid shrimp. Eye stalk ablation has been used to mature female shrimp in captivity [8-10]. Eyestalks are the endocrine center for regulating many physiological mechanisms such as molting, metabolism, sugar balance, heart rate, pigments and gonad maturation. Therefore, unilateral eyestalk ablation affects all aspects of shrimp physiology. Predictable induced reproduction in captive penaeids without the use of eyestalk ablation was considered a long term goal for shrimp aquaculture [11,12].

Various alternatives to ablation have been evaluated, based on accumulated knowledge about environmental control and crustacean endocrinology. Photoperiod and temperature manipulations based on seasonal natural variations of these parameters have been successful in controlling maturation of unablated *P. japonicas, P. stylirostris* and *P. setiferus* [13-15]. However, photoperiods control seems to be more important for subtropical species for review: [3]. In this study, we compared spawning success and nauplii production of broodstocks source in imported SPF broodstocks with historical reproductive performance data from broodstocks are reared in the maturation tanks in order to determine if reproductive performance is compromised under bio secure conditions.

Material and Methods

This study was carried out from October 2012 to November 2013 in M/s Lotus aqua hatcheries, Chennai, India. Where suitable research facilities for the study of hatchery operation and management were ready available.

Broodstock

The broodstocks were imported from SIS (Shrimp improvement system) Florida, USA and quarantined by the Aquatic Quarantine Facility (AQF) to ensure the SPF status of the imported broodstock, as a consequence avoiding the permission of any infested broodstock into the region. The matured male and female were packed at 2 no's of the individual bag with proper oxygenated by the insulated vehicle before carried to the hatchery. The broodstocks transport during the night time is avoided for the stress. Keep rubber tubes cover the rostrum of the shrimp to evade puncturing the plastic bags (Figures 1-3).

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Figure 1: Ready to spawn in *L. vannamei*.

Figure 2: Matured Female in *L. vannamei*.

Figure 3: Matured male in *L. vannamei*.

Broodstock maturation techniques

The matured male and female broodstocks are reared separately. Each maturation tank was 6 × 7 metres in size and 1.2 metres in depth. Each tank can hold up to 200 pieces of broodstock (at a stocking density of 5/m^2). The maximum water level should not higher than 70 cm, to keep 50 cm from the water surface to the top of the tank to avert the broodstock from jumping out. Prepare the water for stocking the new broodstock by pumping water from the 1st Stocking tank through 1 micron Filter bags (4-5 layers) to the broodstock maturation tank until you get 30-40 cm water depth. Adjust the temperature to 27°C by a chiller. The broodstock should be 7 months of age and the males >35 g average weight, while females >40 g average body weight. Stock the males into the male tanks and the females into the female tanks with 200 pcs/tank.

Fresh foods for maturation

Only fresh broodstock feeds is used (such as polychaetes, squids, oyster and INVE Semi-moisture pellet feed) and this is supplemented with feed additives to improve their stage of maturity.

Experimental design

Each maturation tank was painted in black and had a central outlet. The drained sea water was recirculating through bio filters, cartridge filter, activated carbon filter and protein skimmers. Recirculation rate was adjusted to 1200% of each tank volume per day. In addition, 5-10% fresh seawater was supplied to recirculation system to avoid high nitrate concentrations. Fluorescent bulbs 80 W were hung 0.5 m above each tank to obtain the desired photoperiod 14 h light and10 h dark. Molting, maturation and spawning of each individual female were monitored and recorded daily. For this purpose, females were marked by number tagging around the eyestalk.

Evaluated parametres

Water quality: Temperature, total ammonia, pH and dissolved oxygen were measured daily using test kits (Merck, Germany) Salinity and nitrite measured weakly.

Eyes stalk ablation: Prepare the ablation equipment, 3-4 pcs ablation forceps, gas burner (LPG), gloves, antiseptic (acriflavin solution), broodstock cage, etc. Exchange 100% of the water in the Female tank one day before ablation and check to make sure that the females are all intermoult and have hard shells (Molting or soft females will die if ablated). Reduce the tank water level down to 30 cm. Collect all the females in a collection cage. Heat the tip of the forceps with the burner until red-hot. Then carefully hold the female and squeeze the eyestalk of one side with the heated forceps tip. Smear the injured eyestalk with acriflavin solution and release the animal in to the tank. Repeat for every female in the cage. Count and record the number of females in the tank [16].

Spawner's source and mating: About one week after ablation, some females were become stage 4 and be ready for mating and spawning. In the Male tank which is used for mating is conducted at 90-100% water exchange before adding the ripe females because this tank needs to be clean with clear water for mating. Start to select only those females with stage 4 ovaries at 3.00 pm and place them into the male tank. Normally we can get stage 4 female at about 10% of the total females in the tank daily. Record the number of stage 4 females which are transferred to the mating tank (male tank). During the mating period (3-10 pm), turn on the lights over the mating tanks [17].

Spawning and hatching of the broodstocks: Collect the mated gravid females (those that have 2 sperm sacs contacting the thelycum of the females) from the mating tank (male tank). The gravid spawners were dipped for about minutes in 100 ppm formalin. After formalin bath the female was rinsed with sea water and placed into the spawning tanks. In each spawning tank 500 L water treated with 10 ppm EDTA to bind possible heavy metals and 0.1 ppm treflan for fungicides and placed a gravid female for spawning.

After spawning the spent females were removed from the tanks by a scoopnet. The tank water was drained and the eggs were passed through a 350 micron hand net which retains faeces and they were collected on a 100 micron net in a harvest bucket. Before transferring the eggs to the hatching tanks, they were washed thoroughly with running sea water at least for 5 minutes and then they were treated with 100 ppm formalin for 30 seconds and 50 ppm iodine for 60 seconds and again washed thoroughly with running sea water for 5 minutes before being placed into hatching tanks 500 L. For further 36 h to determine hatching rate. The number of eggs and the percentage of the fertilized eggs were estimated by using the formula of [18]. The hatching rate was determined by using the formula

$$H\% = \frac{Y}{X^{*}100\%}$$

Where

H=Hatching rate,

Y=Total number of nauplii and

X= Total number of eggs [19].

Selection of nauplii

At 5.00 am in the morning of the following day (the day after hatching), harvest the nauplii from the hatching tank and put into the nauplius collection tank. Harvest the nauplii by installing the nauplius harvesting net to the hatching tank's outlet pipe. Fill the harvesting channel with clean sweater until it overflows. Now the water level in the channel will be half of hatching tank level. Slowly open the tank valve to release the water with nauplii into harvesting net in the harvesting channel. Adjust the water level in the harvesting channel to control the strength of water current (from the pipe), so that it is not too strong.

Reproductive performance

An important aspect of the present study is on the influence of no. of spawning condition on the growth and maturation of shrimps under captivity. This study is aimed in filling up the gap in aquaculture industry. Naupli production is a function of the number of eggs per spawning. As the size of females increased from 30 gm to 52 gm in (Table 1), the mean number of eggs per spawning increased from 150,000 to 400,000. Although females were increasingly larger through the study period (Table 1) and the larger females would be expected to produce more eggs per spawning and hatching. The relationship between eggs per spawning and the spawning number differed between no. of spawning and body weight of broodstocks. The hatching rate was increased from 3rd spawning onwards. The nauplii production rate (NPR), which is a function of egg production and hatching rate, were significant increased from 15,000 to 222,000. The lower hatch rate for 1st to 3rd spawning depressed the value for the NPR.

Result and Discussion

Eye stalk ablation is still the most effective and common method used for the induction of ovarian maturation in penaeid shrimps. As with other species [3,20-22] the eyestalk ablation was found to be the best technique in the maturation and spawning of the pacific white shrimp *L. vannamei*. In agreement with [21,22] the eyestalk ablation generated more spawning and egg-production, but higher fertilization or hatching rates were increase in our present study (Table 1 and Figure 4).

Size of maturation tanks and brood stock stocking density are known to influence mating's and ovarian development in shrimps. A study carried out with the green tiger shrimp *P. semisulcatus* had good results 1.2 m diameter tanks at 1:2 male/female ratio and 10 shrimps per m^2. In this study, we found similar reproductive performance (spawning rate, fecundity, fertilization and hatching rates) [23-25].

In our present study, each tank is 6 × 7 metres in size and 1.2 metres deep. One tank can hold up to 200 pieces of brood stock at a stocking density of 5/m^2 produced significantly more eggs per female and hatching rate 90% were found. Based on our results and those at the literature [20,24], it can be concluded that brood stock tanks of not smaller than 3 m in diameter have to be preferred for the successful reproductive performance of *L. vannamei*. [19] suggested the use of at least 6 m^2 of the tank bottom for *L. vannamei* brood stocks.

In general, fertility rates were high but hatching rates were unexpectedly low. Many factors such as low water quality, inappropriate photoperiod, insufficient quantity or quality of the feeds or even genotype of the broodstocks might account for low hatching rates [26]. It is well known that nutrition is one of the main factors influencing gonad development in shrimps (Table 2). In commercial hatcheries, broodstocks are generally fed on fresh seafood (mussel, oyster, squid, crab or sea worms) and sometimes artificial feeds until satiation for successful maturation and spawnings [27-30]. Similarly, in our study we also fed the broodstocks on fresh and occasionally on frozen Polychaetes, squid, oyster, green mussel and commercially INVE

No. of spawning	Gms	No. of Eggs (× 1000)	Nauplii (× 1000)
1	30	1.5	0.18
2	31-32	1.62	0.26
3	32-33	1.68	0.50
4	33-34	1.73	0.74
5	34-35	2.18	1.03
6	35-36	2.42	1.26
7	36-37	2.84	1.65
8	37-38	3.12	1.96
9	38-40	3.51	2.56
10	40-42	3.74	3.30
11	42-44	3.82	2.53
12	44-46	3.93	1.63
13	46-48	4.11	1.33
14	48-50	4.23	0.83
15	50-52	4.42	0.61

Table 1: Relationship between body weight, eggs and nauplii in *L. Vannamei* spawning.

Figure 4: Induce Eye stalk ablation in *L. vannamei.*

Feed Time	% of Feed	Combination
07.00 am	15	Feed with Polychaetes.
11.00 am	10	Feed with Squids.
16.00 am	10	Feed with Oyster.
22.00 am	60	Feed with **Polychaetes.**
02.00 am	5	Feed with INVE Semi-moisture pellet feed.

Table 2: Feeding program for broodstock.

Breeds semi moisture pellet feed to provide nutritionally balanced diet (Table 2).

The predominant feeding practice in shrimp hatcheries and in shrimp broodstock feeding trials has been used fresh feeds such as annelid worms (polychaetes), squid, bivalves (mussels, clams and oysters), crustaceans (shrimp, crab, artemia) and fish. Feeding of polychaetes can enhance reproductive performance of shrimp due to their high nutritional composition, high unsaturated fatty acid content (e.g., arachidonic acid) and the presence of reproductive hormones [31]. Polychaetes are not only an excellent source of HUFA but possibly also a good source of reproductive hormones, similar to those found in shrimp. To date, reproductive hormones of polychaetes such as progesterone (P4) and 17alpha-hydroxy progesterone have been reported to be capable of inducing shrimp oocyte development [32]. In addition to water temperature, photoperiod, light intensity and quality, many other water quality parametres i.e., salinity, pH, dissolved oxygen, nitrogenous wastes, heavy metals etc might also influence spawning success in penaeid shrimps [6,33]. In order to ensure best water quality, we recirculate the seawater through biological filter and UV systems prior using in the spawning tanks and hatching tanks. EDTA, an antibiotic oxytetracycline and povidone iodine were also used to chelate heavy metals and control infections [34,35]. As a result we never observed any kind of disease throughout the experiment. The photoperiod, temperature, salinity, pH and all other environmental parametres were adequate for good hatchery practices (Table 3).

"Domesticated stocks from Colombia (Wt 37 g) eggs/female 105,000 (n=25), Wild stocks from Panama (pond raised from wild nauplii kept in maturation tanks with (Wt 29.5 g) eggs/female 116,000 (n=25), Ecuadorian (wild broodstock kept in maturation tanks with (Wt 60 g) eggs/female 230,000 (n=130)." Other references showing higher egg numbers with larger animals are [5,36] showed that wild kuruma shrimp broodstock produced about the same number of eggs as equal sized domesticated kuruma shrimp broodstock, but the survival of the domesticated stock larvae was half that of wild.

In general the use of pond-reared broodstocks in the hatcheries results in lower reproductive performance compared to the wild counterparts [37]. Reported serious larval quality problems from the domesticated broodstock of P. japonicus or L. vannamei both of which are considered to be relatively easy shrimp species to reproduce in captivity. It is also know that inbreeding depressions might often be seen when pond reared animals are used as broodstock for several generations [38]. When a new species imported into a country and its life cycle is closed, the reproductive performance might seriously be hampered due to low genetic variation [39]. In fact, in the breeding programs with low genetic variation, inbreeding depression might occur even after 1 to 2 generations. [40] found 33.1-47.1% lower hatching rates in *L. vannamei* due to inbreeding depressions even after two generations. These researchers reported that the stress exerted by environmental parameters worsen the situation further. As a result, the high fertility and hatching rates we obtained in the current study might have been influenced by the good reproductive performance owing to captive conditions, season as well as genotype of the brood stock of L. vannamei (Figure 5).

Conclusion

Our present findings gave adequate outcomes on ovarian maturation and spawning in imported *L. vannamei* of Specific Pathogen Free (SPF) shrimp broodstocks in captive eyestalk ablated spaweners, among connection between body weight, eggs and Nauplii. The results

Parameters	Range
Temperature	27.5-28.5ºC
Salinity	33ppt
Total ammonia	0-0.5
Nitrate	0-0.3
pH	7.8-8.2

Table 3: Levels water quality parameters in *L.Vannamaei* Maturation.

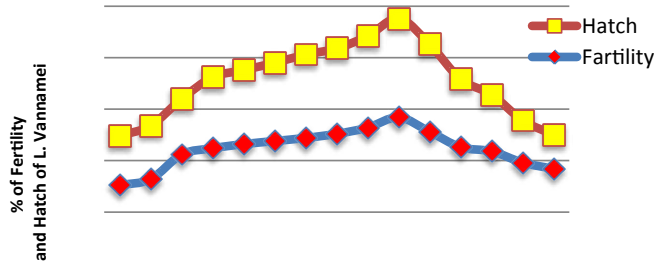

Figure 5: Rate of Fertility and Hatching of *L. vannamei* spawning.

of this study has demonstrated that under Mediterranean climatic conditions, the broodstock of this non-indigenous shrimp species can be readily matured and spawned out of season in recirculating matured and spawned out of season in recirculating systems. However, further research is required to increase the spawning activity and evaluate the duration of the reproductive performance in *L. vananamei* and also has to be carried out to improve hatching rate and nauplii production. To create additional better mixtures of feeds which will supportive for the production of high yield nauplii by way of hatchery post larval production is concerned. This way is useful absolutely achieve the gaps in shrimp industry.

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