

# Reproductive Performance of Wild Brooders of Indian White Shrimp, *Penaeus indicus*: Potential and Challenges for Selective Breeding Program



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## ABSTRACT

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Indian white shrimp, *Penaeus indicus*, has been identified as a national priority species for domestication and genetic improvement. Although breeding and farming of this species has been studied before the inception of commercial shrimp farming in India, reproductive and hatchery performance of this species on a mass scale has not been addressed so far. To evaluate the reproductive performance of wild *P. indicus* brooders, a total of 2164 brooders from the broodstock fishery along the Indian east coast were used. The experiment was carried out in two phases; in trial 1, brooders from Odisha, Kanyakumari, as well as Chennai, were used, whereas, in trial 2 brooders from Chennai coast alone was used. Only 16-32% of eyestalk ablated animals spawned successfully, whereas remaining stock was found to be nonresponsive to eyestalk ablation. Ablated females had a latency period of 7-10 days with 2-3 times spawning per brooder. The average fecundity of wild spawner was 220000±56000. Eggs per gram body weight for wild and ablated spawners were 8126±3502 and 1481±863, respectively. The egg hatchability was 80% for wild spawners whereas ablated spawners recorded 50-70% hatchability. During larval rearing cycle a lack of synchronized moulting was noticed during protozoa to mysis conversion (91.5% protozoa and 8.5% mysis 1), and mysis 3 to postlarvae (PL) conversion (30-50% of mysis 3 in PL1/PL2 stage). The study provides a deeper understanding of the reproductive performance of wild broodstock of native *P. indicus*, which can be used as a reference database for future breeding programs.

**ADDITIONAL INDEX WORDS:** Broodstock, closed thelycum, eyestalk ablation, length weight, *Penaeus indicus*, reproduction.

## INTRODUCTION

Shrimp culture being the highly productive aquaculture sector, forms a significant share of the export revenue of many Asian countries. At present, the global shrimp industry is dominated by Pacific white shrimp, *Penaeus vannamei* with a lion share of 86% to the total shrimp production, 4.8 million metric ton (FAO, 2018). Expansion of *P. vannamei* culture across the world owes to availability of fast-growing and specific pathogen free (SPF) strains, resulting from the selective breeding program. These made the species *P. vannamei* to have multiple advantages such as wide tolerance to environmental characteristics, better ability to utilize low-protein diets, ability to readily reproduce in captivity and fastest growth rate compared to other penaeid shrimp species (Wyban, 2007).

Indian white shrimp, *P. indicus* is the first indigenous penaeid shrimp seed production standardized in India during the

1980s (Muthu and Laximinarayana, 1982). Although scientific shrimp farming of *P. indicus* was initiated in early 1980, when aquaculture of tiger shrimp, *P. monodon*, was popularized, this species has lost its importance. However, rampant white spot syndrome virus (WSSV) epidemics since 1994-95 caused farmers to leave the shrimp industry or to take up shrimp culture with high speculation. In this situation, the introduction of exotic species SPF *P. vannamei* in 2009 gave impetus to shrimp farming. Although SPF *P. vannamei* farming resulted in spectacular growth to the Indian aquaculture industry, its burgeoning culture across the country without emphasis on sustainability has resulted in the emergence of new diseases [e.g., early mortality syndrome (EMS), *Enterocytozoon hepatopenaei* (EHP) etc.]. In this scenario, it became highly imperative to explore and revive the native stocks of penaeid shrimps in India, and *P. indicus* forms an ideal alternative to achieve this goal (Vijayan, 2019).

Many researchers studied hatchery production and biology of reproduction of *P. indicus* during the pre WSSV era (1980-90s) in India (Mohamed and Diwan, 1991; Muthu, 1980; Vijayan, 1989), South Africa (Emmerson, 1980), Philippines

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(Primavera, 1985) and Israel (Mosha and Gallardo, 2013). At present, the majority of brooders are WSSV carriers, and screening of brooders to select disease-free broodstock becomes a major challenge. A recent survey on the prevalence of WSSV among wild-caught brooders in the Indian coastline indicated a high prevalence of the virus among *P. monodon* and *P. indicus* brooders (Chakrabarty *et al.*, 2014; Debnath, Karim, and Belton, 2014; Vijayan, 2019). In this scenario, it is highly pertinent to develop disease free domesticated broodstocks as a preliminary step to raise diseases resistant and SPF broodstock suitable to tropical environmental conditions in India. To achieve this goal, a detailed study of the inherent reproductive performance of existing feral stock becomes highly relevant.

Although several reviews on the maturation of penaeids have published over the past few years (Alfaro-Montoya *et al.*, 2016; Arcos, Racotta, and Ibarra, 2004; Bray and Lawrence, 1992; Browdy, 1998; Diwan, Harke, and Panche, 2018), information available on the reproduction of closed thelycum shrimps are highly dispersed (Coman *et al.*, 2013). There is a scarcity of data on reproductive performance of wild *P. indicus* broodstocks in a near commercial system following two decades of WSSV prevalence. The findings from the present study can provide a further basis for the selection of broodstock to achieve best spawner performance. Against this background, the objective of the present study was to summarize the current knowledge on the maturation and reproductive performance of *P. indicus* from the Indian coast, which can improve sustainable seed production in India.

## METHODS

In this paper, two trials were designed to assess the reproductive performance of *P. indicus* brooders from different locations along the Indian coastline *viz.*, Odisha, Chennai, and Kanyakumari (Trial 1) during the year 2015, and brooders along Chennai coast during the period 2015-2017 (Trial 2).

### Experimental Broodstock

Male and female brooders of *P. indicus* were obtained from trawl and gillnet fishery in the Bay of Bengal from Kanyakumari to Odisha coast, India. Though life history and age composition of the species in each season was not known, broodstocks having different stages of maturation were collected. Wild brooders were transported in brooder bags (3.5 L) with rubber tubes around the rostrum to avoid puncturing the broodstock bags. The number and size of the male, female brooder, seasons etc. are given in Table 1.

### Quarantine and Disease Screening

Experimental brooders were slowly acclimatized based on temperature and salinity at the collection site and stocked individually in 100 L FRP tanks after treating with formalin dip (200 ppm) in quarantine facility at Muttukadu Experimental Station, (MES) of ICAR-CIBA, Chennai, India. Brooders were diagnosed individually for WSSV through nested PCR.

### Length-weight, Condition Factor, and Gonadosomatic Index

To understand the length-weight relationship and condition factor of brooders, male and female brooders were individually weighed using a digital electronic balance having 0.01 g

precision and with a standard ruler with an accuracy of 0.1 cm. Total length (TL), standard length (SL) and carapace length (CL) were measured from tip of the rostrum to telson, postorbital notch to the tip of the telson and postorbital notch to posterior carapace margin respectively. The relationships between TL and wet weight (W) were calculated by the power regression  $W = a \times L^b$ . The association degree between total length, standard length, and weight was calculated by determination coefficient ( $r^2$ ). Fulton's condition factor (K) estimated from the equation,  $K = 100 W/L^b$ , where K = condition factor, W = mean weight (g), TL = body length (cm), and b value were derived from the  $W = a \times L^b$ . Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as per the given formula:

$$\text{Gonadosomatic index (GSI)} = \text{Ovary weight (g)} / \text{Total body weight (g)} \times 100 \quad (1)$$

$$\text{Hepatosomatic index (HSI)} = \text{Hepatopancreas weight (g)} / \text{Total body weight (g)} \times 10 \quad (2)$$

### Broodstock Maturation

Healthy WSSV-free brooders were stocked in the maturation facility at MES of CIBA, Muttukadu, Chennai (India). Each shrimp was individually tagged on the eyestalk with a numbered, plastic ring for identification and stocked in maturation tanks (5 ton; 8 m<sup>2</sup>) with seawater (30-33 g L<sup>-1</sup>). A photoperiod of 18D: 6L was maintained with the help of fluorescent bulbs (80W). Temperature, total ammonia nitrogen (TAN), pH, nitrite-N were measured during each cycle as per the standard protocols (APHA, 2012). After acclimatization, brooders at intermoult/late premoult were unilaterally eyestalk ablated. Ablated females were reared with males at 1:3 ratios in maturation tanks at a density 3 numbers / m<sup>2</sup>. Broodstock diet consisted of frozen squid, polychaetes, bivalves, and pelleted maturation diets (INVE Aquaculture, Inc. USA). The broodstocks were fed with maturation diet at 25% live wet weight at four times per day. Daily ration for fresh feed was 5 to 10%, and pellet feed was 3% of body weight. Water temperature and salinity were maintained at 28-30°C and 30-33 g L<sup>-1</sup> respectively, and 100% water exchange per day was followed to maintain minimum nitrogenous metabolites (TAN, NO<sub>2</sub>, etc.). Females were periodically examined for ovarian maturation by shining a light beam through dorsal exoskeleton under dark condition. Ovarian tissue of female at different gonad development stages was dissected out, fixed in 10% neutral buffered formalin for a period of 24 h, and then transferred to 70% alcohol for histological analysis. Histological analysis was carried out according to the procedure suggested by Bell and Lightner (1988).

### Spawning and Seed Production

Females at ovary stage late III (maximum) and IV based on the visual observations (Tomy *et al.*, 2016) were transferred individually to spawning tanks (500 L) in the late evening and allowed to spawn. Spawning tanks were filled with filtered seawater, and EDTA (10 ppm) was applied as a chelating agent to remove heavy metal from seawater. Spawning generally happened at 2: 00 to 3: 00 and spawned females were transferred

Table 1. Morphometric parameters of brooders (mean±SD), collection site, and the number of spawning cycle performed during the study period.

Parameters	Trial 1			Trial 2			
	2015			2015-16		2016-17	
	Chennai	Odisha	Kanyakumari	Chennai	Chennai	Chennai	Chennai
Study period	Pre-monsoon	Pre-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-Monsoon
Number of brooders collected	280	230	218	260	440	335	401
Number of successful trials	5	3	4	3	4	0	2
Length of female brooders (cm)	17.2±1.4 (14.5-21.0)	15.5±1.0 (14.5-17.8)	15.4±1.6 (12.0-20.0)	15.6±1.6 (12.8-19.8)	15.83±1.56 (12.7-20.5)	15.21±1.5 (12.5-20.5)	15.79±1.9 (14-19.8)
Weight of female brooders (g)	64.3±4.1 (58-70)	53.9±8.9 (39-65)	58±10.8 (31-82)	35.67±12.8 (20.4-71)	39.01±12.58 (21-91)	36.93±10.5 (20.5-91)	39.63±11.75 (27.7-74)

Ranges are given in parenthesis.

back to maturation tanks 4-5 h after spawning. Collected eggs were washed through gentle running seawater for 5 min, followed by dip treatment in treflan (0.01 ppm) and povidone-iodine (100 ppm) for 30 sec and transferred to hatching tank. The egg diameter, fecundity, and hatchability were documented. Fertilized eggs were hatched out at 10-12 h interval, and slight aeration and periodic shuffling were provided to facilitate hatching. Four subsamples were taken 2 to 3 h after first hatching to estimate the total number of nauplii. Positively phototactic nauplius, N-5 was collected, and dip treatment of formalin (100 ppm) and treflan (0.1 ppm) were given for 30 sec followed by 5 min wash with steady, but a slow current of clean seawater. Fecundity, percentage of fertilized eggs, hatching rate, were estimated using the formula given below:

$$\text{Fecundity} = \frac{\text{Total number of eggs released}}{\text{number of brooders}} \quad (3)$$

$$\text{Eggs per gram body weight} = \frac{\text{Total number of eggs released}}{\text{body weight of brooder (g)}} \quad (4)$$

$$\text{Percentage of fertilized eggs} = \left( \frac{\text{Total number of fertilized eggs}}{\text{total number of eggs}} \right) \times 100 \quad (5)$$

$$\text{Hatching rate} = \left( \frac{\text{Total number of hatched Nauplius}}{\text{total number of eggs}} \right) \times 100 \quad (6)$$

### Larval Rearing and Feed Management

The nauplius (N5) was stocked at 100 no. / L or 100000 / ton in larval rearing tanks (LRT) after treating with EDTA (10 ppm) to chelate heavy metal and treflan (0.1 ppm), an antifungal agent. Vitamin C and probiotic supplements were applied at 1 ppm daily. Microalgae like diatoms, *Chaetoceros* sp. was used for feed management for Zoea1 to 3 stages, and *Skeletonema* sp. was provided from stage 1 mysis onwards at a concentration of 80,000-1.1 lakh cells/ml. Alternatively, dried *Spirulina* and commercial microencapsulated diets with different sizes (50-120; 120-250 and 250-500 µm) FRIPPAK, INVE Aquaculture, Inc. (USA) was used for protozoa, mysis, and PL stages as per manufactures suggestions. Mysis 3<sup>rd</sup> stage onwards disinfected live artemia nauplii instar1 were provided at 1-4 no./larvae till PL10 along with the commercial microencapsulated feed. Each larval stages were examined under the microscope to record larval conversion stages, larval deformity and zoea conversion problems (lack of feed intake of zoea 1 stage and its conversion

into zoea 2) etc. Mysis 1 onwards, daily water exchange was done at 30-50% per day using 250-500 µm filter bag.

### RESULT

A total of 21 seed production trials, each of about 30-40 days duration were carried out from April 2015 to 2017. The average size of male and female broodstocks collected was 39.01±12.58 g with size range 21 to 91g and carapace length 2.8 to 5.3 cm indicates different age groups of the brooder. In the year 2015, the WSSV prevalence was below 10-30% whereas, in the year 2016-17, its prevalence among the brooders went up to 70-90% in pre and post-monsoon period and was found to be 45% in the post-monsoon period.

### Reproductive Performance

Evaluation of the reproductive performance of Indian white shrimp collected from different coastal sites, Chennai, Kanyakumari, and Odisha during the pre-monsoon period (Trial 1) revealed that only 12.8-32.5% brooders participated in spawning with brooders collected from Chennai coast contributing maximum percentage in spawning (32.5%). Similarly, brooders collected along the Chennai coast during the year 2015-17 (Trial 2) also revealed that only 16-32% of ablated spawners contributed to spawning whereas 78-84% of ablated broodstock remained non-responsive (Table 2). Ablated females had a latency period of 7-10 days, and more than 80% of the ablated shrimp attained only the 3<sup>rd</sup> stage of ovarian development during spawning. The egg quality and larval survival of wild spawners were found to be higher compared to ablated spawners. Spawners belonging to lower size group had higher eggs per gram body weight and followed an inverse relationship with the size class (Figure 1).

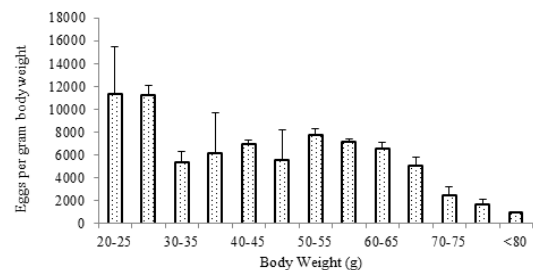


Figure 1. Eggs per gram body weight (mean±SD) of *Penaeus indicus* brooders spawned during the study period.

Table 2. Reproductive performance (mean±SD) of wild *Penaeus indicus* brooders during the study period.

Performance parameters	Trial 1			Trial 2
	Chennai	Odisha	Kanyakumari	Chennai
Ablated spawners contributed in spawning (%)	32.5±8	12.8±1.3	27.6±8	25±4
Fecundity of ablated spawners	148000±92000	160000 ± 80000	180000±70000	65000±16000
Fecundity of wild spawners	-	-	-	220,000±56000
Eggs/gram body weight of ablated spawners	3894±783	2739.9 ± 1529.7	3261±1513	1481±863
Eggs/gram body weight of wild spawners	-	-	-	8126±3502
Gram/weight ratio of wild brooders	3.7±1.4	3.48±0.62	3.67±0.58	2.38±0.49

The egg hatchability of wild spawners was above 80% whereas ablated spawners recorded 50-70% hatchability, and eggs hatchability was observed to reduce following successive spawning (2-3 no. per spawn). Similarly, the percentage of nauplius contributed by wild spawners from the Chennai coast was 27% whereas 100% nauplius produced from Odisha and Kanyakumari was from ablated spawners.

Gonadosomatic index (GSI) of the female population at different stages of ovarian development ranged between 0.9 and 12.7%. The gravid ovary was visible through the exoskeleton, and it occupied all the available space in the body cavity (GSI = 6-12.7%) during the 3rd and 4th stage of ovary development (Figure 2). Light green to deep olive greenish colour was observed in the 3rd and 4th stages of ovary development. Histology of stage 4 ovary revealed large (160-230 µm) acidophilic oocytes with cortical rods in the periphery of the cytoplasm (Figure 3a-e). Presence of atretic oocyte was the primary distinguishing character of spent ovary compared to the first stage though morphology and colour of the ovaries were very similar and GSI were within 1.1-2.0%. Previtellogenic oocyte (< 50 µm) or immature cells were predominant before the onset of vitellogenesis and were basophilic.

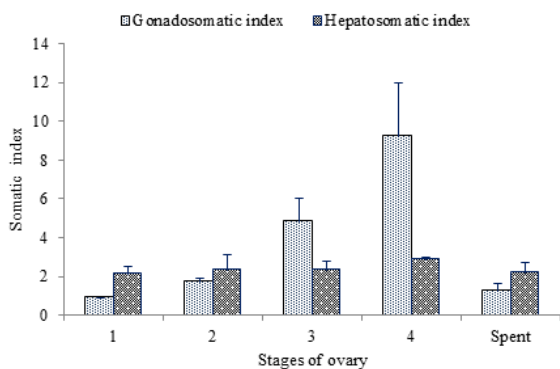


Figure 2. Gonadosomatic and hepatosomatic index (mean±SD) of *Penaeus indicus* brooders at different reproductive stages during the study period.

Yolky acidophilic oocytes were recorded in the early to late vitellogenic phase (50-150 µm) due to vitellogenin accumulation in the cytoplasm. The salinity during the seed production study was 29.2 to 30.8 g L<sup>-1</sup>. Total ammonia nitrogen (TAN) level in maturation and larval rearing tanks were tanks 0.77±0.04 and

0.09±0.02 ppm respectively, and total nitrite-N level is 0.11±0.14 and 0.03±0.01 ppm, respectively.

#### Length-weight Analysis and Condition Factor

Length-weight analysis of female and male brooders resulted in the equation  $Y=0.03 \times 2.6$ ;  $R^2=63$  and  $Y=0.05 \times 2.4$ ;  $R^2=71$ , respectively, which indicates female and male brooders exhibit negatively allometric growth curve where the length of the brooders does not increase proportionately with weight (Figure 4). Fulton condition factor which indicates animal wellbeing or overall productivity based on total length and the standard length was 0.98±0.20 and 1.56±0.30 respectively for females with the highest value noticed in pre-monsoon periods (1.65-1.67).

#### Larval Performance

The life cycle of *P. indicus* larvae has three larval stages, nauplius, protozoa (1, 2, 3) and mysis (1, 2, 3). Each larval sub-stage took an average of 1.5 to 2 days for conversion. The size range of eggs and larvae in comparison to previously published data for *P. indicus* larvae (Muthu, Pillai, and George, 1978) is given in Figure 5. Lack of synchronized moulting was one of the major problems noticed during larval rearing. During the conversion from protozoa to mysis, a mixed larval stage, *i.e.*, 91.5% protozoa and 8.5% mysis I was noticed. Maximum lack of synchronized molting was recorded during mysis 3 to PL conversion stages; *i.e.*, 30-50% of mysis 3 stages during PL 1/PL 2 conversion stages which indicate a high chance of cannibalism among *P. indicus* larvae. Nauplius to mysis survival was recorded as 60% (50-70%) in a successful larval cycle, and average zoea conversion mortality varied among the cycle (5-33%), and was highest in ablated females and lowest in wild brooders.

#### DISCUSSION

Reproductive behavior of penaeid shrimp varies within and between the species (Dall *et al.*, 1990). Reproductive performance of wild brooders revealed that only 16-32% of the population participated in spawning wherein a maximum of 2-3 spawning per brooder was observed. *P. indicus*, being a closed thelycum species, mates after each ecdysis as spermatophore is generally lost with the exuviae (Makinouchi and Primavera, 1987). It is reported that eyestalk-ablated impregnated *F. paulensis* females are not affected by the presence or absence of males in maturation tanks (Peixoto, Cavalli, and Wasielesky,

2005) as gonad development start after impregnation. Though ovarian development prevents moulting; onset of moulting noticed in impregnated wild brooders immediately after eyestalk ablation in the present study became a constraint due to lack of successful impregnation and further maturation. During ecdysis, resorption of the ovary was also noticed which corroborate with earlier reports (Emmerson, 1980). It is widely documented that ovaries of many induced female populations either never mature or have prolonged latency period (Arcos, Racotta, and Ibarra, 2004; Bray, and Lawrence, 1992; Palacios, Ibarra, and Racotta, 2000) and eventually leads to deterioration of spawn quality (Emmerson, 1980; Harison, 1990; Palacios and Racotta, 2003). These type of inherent reproductive constraints such as poor mating is reported both in open and closed thelycum penaeid breeding population (Arcos, Racotta, and Ibarra, 2004). In the current study, the male: female ratio of 1:3 maintained, and maturation tanks used were neither so large nor circular.

Though it is reported that poor mating success can be associated with the size and shape of maturation tanks (Peixoto, Cavalli, and Wasielesky, 2005), as females tended to stay on the bottom during copulation, tank size may not have played a significant role. However, provision of the sand base has been reported to enhance impregnation of *P. monodon* brooders in maturation units which demand modification of maturation units to simulate natural condition to enhance impregnation (Arnold, Coman, and Emerencano, 2013).

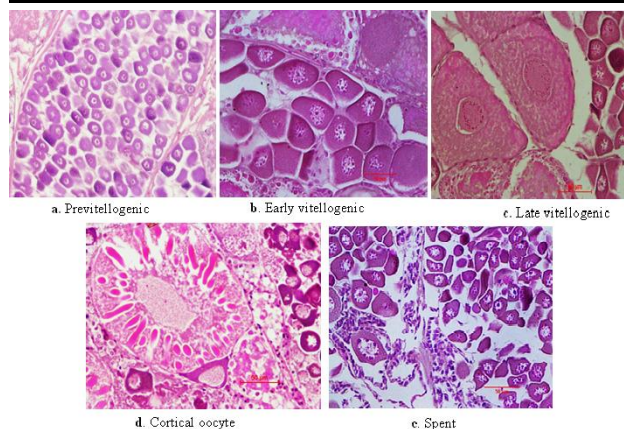


Figure 3a-e. Transverse sections of different stages of *Penaeus indicus* ovarian tissues stained with hematoxylin and eosin (40 X).

In the present study, ablated shrimp had a latency period of 7-10 days. About 11-14 days latency period, high variation in hatching rate (16-97%) with a fecundity of 10,000 to 1.6 lakh was reported for *P. indicus* (Muthu and Laxminarayana, 1982). According to Makinouchi and Primavera (1987), wild ablated *P. indicus* females (11.5±3.1 g) had a latency period of 4-6 days. The fecundity of Indian white shrimp reported in the present study was much higher compared to previous reports (Emmerson, 1980; Muthu, 1980; Primavera, 1985) which might be due to use of pond-reared shrimps with smaller size groups in earlier studies, whereas wild brooders of different size groups were a part of this study.

Moreover, as the majority of the brooders procured were impregnated, the number of spawning each brooder had in the

wild was not known. Although pond reared brooders to exhibit greater survival in a captive system, the reproductive performance would be poor compared to the wild counterparts (Benzie, 1997).

Though eyestalk ablation gives predictable peak of maturation, deterioration in quality of spawn and quantity over time period is reported either due to stress associated with ablation procedure (Palacios, Ibarra, and Racotta, 2000) or reduction in nutrient reserve in ovary (Harrison, 1990; Primavera, 1985), and observations noted in this study are in line with these findings. Many times, rapidity of ovarian development in ablated female results in rushed spawning where eggs may not be at the final stage of development. However, recent reports of SPF *P. vannamei* spawning performance in commercial hatcheries in India revealed a higher fecundity and nauplii production from successive spawning compared to that of the initial spawning (personal communication), and its quality remains more or less similar up to 8-10 spawning with the highest spawning frequency of 10-12 numbers per brooder (Kannan *et al.*, 2015). These observations were further reinforced by the earlier studies on egg biochemical composition, and larval quality analysis that remained unaffected by the multiple spawning in *P. vannamei* (Arcos, Racotta, and Ibarra, 2004; Palacios *et al.*, 2001). As the majority of these works are focused on selectively bred or domesticated stock of open thelycum shrimps with better broodstock performance ability and tracked spawning history unlike wild spawner where the number of spawning already happened is oblivious. These indicate the need to develop domesticated strains with superior quality maturation diets and highly evolved manipulation techniques (Alfaro-Montoya *et al.*, 2016) to improve the reproductive performance of closed thelycum shrimps.

Reproductive performance of hatchery broodstock is also affected by photoperiod, water quality parameters such as temperature, salinity apart from broodstocks age (Browdy and Samocha, 1985; FAO, 2007; Harison, 1999). Though 100-400% water exchange is recommended in maturation tanks (Bray and Lawrence, 1992), flow through the model with 100% water exchange was followed in the present study as nitrogenous compounds were below the critical level (Cavalli, Scardua, and Wasielesky, 1997). Compared to flow through the system, recirculatory maturation models have better water quality maintenance and retention of pheromones within the system, which in turn can enhance the broodstock performance (Otoshi *et al.*, 2003). Recently, along with eyestalk ablation, administration of neurotransmitters (Tomy *et al.*, 2016) and sex steroid hormones (Merlin *et al.*, 2016) are attempted. A non-lethal alternative to ablation such as manipulation of environmental parameters (photoperiod, temperature *etc.*) or endocrine manipulation techniques is worth attempting in this regard (Chamberlain and Gervais, 1984; Feijó *et al.*, 2016).

Reproductive performance of *P. indicus* documented during the early 1980s in Indian subcontinent (Mohamed and Diwan, 1991; Muthu, 1980), South Africa (Emmerson, 1980), Philippines (Primavera, 1985) and Israel (Mosha and Gallardo, 2013) recorded higher percentage of spawning participation, lower latency period and more successive spawning per shrimps compared to present study. High WSSV disease prevalence in

wild brooders and its inadequate response to ablation might be the reason behind reproductive challenges recorded in the present study. A recent review on reproductive performance of *P. monodon* in Bangladesh shrimp hatcheries recorded deterioration of broodstock performance of ablated spawners, poor hatchability, partial spawning, and decline in survival after ESA over the last few years, and a strong positive correlation between year of collection and broodstock mortality (Debnath *et al.*, 2015). The authors hypothesized that the higher percentage of stressed or diseased broodstocks or changes in the environmental parameters, deteriorating post-capture handling practices, exposure to high viral load in the environment might be contributing to high mortality rate and low reproductive performance. Specific pathogen-free stock can make a solution to declining or deteriorating broodstock quality and high WSSV prevalence, and, therefore, emphasize the need to develop a domesticated stock of *P. indicus* for captive maturation.

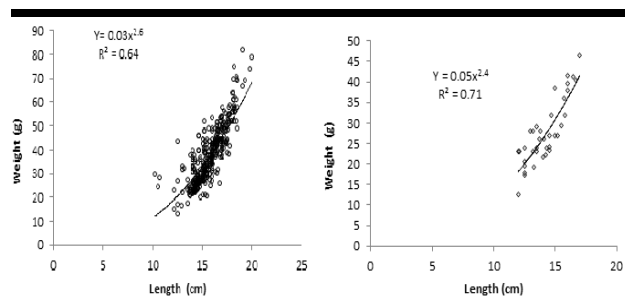


Figure 4. Length-weight analysis of female and male *Penaeus indicus* brooders collected along Chennai coast during the study period.

The growth of penaeid shrimps depends on the sex, developmental stage, and environments (Prasad, 2001). It is reported that *P. indicus* start breeding at 2.7 cm CL (13.7 cm TL) in Madagascar, 3.3 cm CL /15.8 cm TL in S. Africa. The shrimp attain maturity in 5-6.5 months, with 7 to 9 month breeding period (Emmeson, 1980). Length-weight analysis of brooders indicates negatively allometric growth curve where the length of the brooders does not increase proportionately with weight.

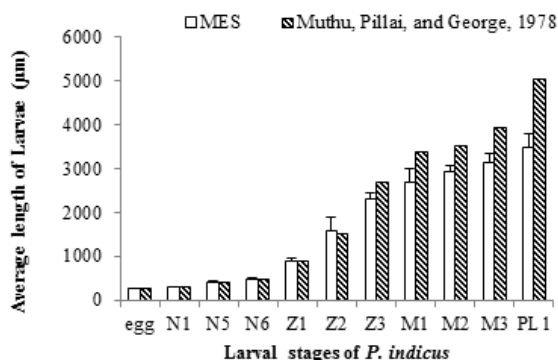


Figure 5. Comparison of the average size of *Penaeus indicus* eggs and larvae compared with earlier reports of Muthu, Pillai, and George, 1978).

Fulton's condition factor (K) used to quantify the animal's physical wellbeing (Gopalakrishnan *et al.*, 2014) concerning total length is noticed below one may be due to water loss or fresh mass loss after spawners. Marked variations in the size of larvae compared to earlier reports (Muthu, Pillai, and George, 1978), and lack of synchronized moulting during the larval conversion was noticed in the present study which can be attributed to high chance of cannibalism among *P. indicus* larvae. These reproductive constraints due to poor broodstock quality, high level of disease prevalence and low responsiveness to eyestalk ablation demand further research for domestication and close the life cycle of closed thelycum *P. indicus*.

## CONCLUSION

In light of the incidence of emerging diseases in *P. vannamei* across the world, it is paramount to revive the native stock, which is suitable for tropical conditions in India. The relative ease in breeding under captive conditions, short period to become reproductive, and comparable growth performance with *P. vannamei* make this species a potential alternative to exotic *P. vannamei*. Seed quality has been the foundation that predicts the success of shrimp, industry and therefore, the present study on the broodstock performance is relevant for the development of captive broodstock population and breeding program. The current findings on its reproductive and larval cycle performance will throw light to understand its basic inherent potential and challenges to revive this species and can pave the way for the development of selective breeding of this species.

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## LITERATURE CITED

- Alfaro-Montoya, J.; Hernández-Noguera, L.; Vega-Alpizar, L., and Umaña-Castro, R., 2016. Effects of androgenic gland ablation on growth, sexual characters, and spermatogenesis of the white shrimp, *Litopenaeus vannamei* (Decapoda: Penaeidae) males. *Aquaculture Research*, 47 (9), 2768–2777.
- APHA, 2012. Standard Methods for the Examination of Water and Wastewater. In: Rice, E.W.; Baird, R.B.; Eaton, A.D., and Clesceri, L.S. (eds.), Washington DC: American Public Health Association, American Water Works Association and Water Environmental Federation, pp. 1300-1496.
- Arcos, F.G.; Racotta, I.S., and Ibarra, A.M., 2004. Genetic parameter estimates for reproductive traits and egg composition in Pacific white shrimp *Penaeus (Litopenaeus) vannamei*. *Aquaculture*, 236, 151-165.
- Arnold, S.J.; Coman, G.J., and Emerenciano, M., 2013. Constraints on seed stock production in eighth generation



- domesticated *Penaeus monodon* broodstock. *Aquaculture*, 410-411, 95-100.
- Bell, T.A. and Lightner, D.V., 1988. *A Handbook of Normal Penaeid Shrimp Histology*, Louisiana, Baton Rouge, World Aquaculture Society, 114 p.
- Benzie, J.A.H., 1997. A review of the effect of genetics and environment on the maturation and larval quality of the giant tiger prawn *Penaeus monodon*. *Aquaculture*, 155, 69-85.
- Bray, W.A. and Lawrence, A.L., 1992. Reproduction of *Penaeus* species in captivity. In: Fast, A.W., and Lester, L.J.(eds.) *Marine Shrimp Culture. Principles and Practices*, Amsterdam: Elsevier, pp. 19-29.
- Browdy, C.L., 1998. Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. *Aquaculture*, 164, 3-21.
- Browdy, C.L. and Samocha, T.M., 1985. The effect of eyestalk ablation on spawning, molting and mating of *Penaeus semisulcatus* de Haan. *Aquaculture*, 49, 19-29.
- Cavalli, R.O.; Scardua, M.P., and Wasielesky, J.W., 1997. Reproductive performance of different sized wild and pond-reared *Penaeus paulensis* females. *Journal of World Aquaculture Society*, 28, 260-267.
- Chakrabarty, U.; Mallik, A.; Mondal, D.; Dutta, S., and Mandal, N., 2014. Assessment of WSSV prevalence and distribution of disease-resistant shrimp among the wild population of *Penaeus monodon* along the west coast of India. *Journal of Invertebrate Pathology*, 119, 12-18.
- Chamberlain, G.W. and Gervais, N., 1984. Comparison of unilateral eyestalk ablation with environmental control for ovarian maturation of *P. stylirostris*. *Journal of World Mariculture Society*, 15, 29-30.
- Coman, G.J.; Arnold, S.J.; Wood, A.T., and Preston, N.P., 2013. Evaluation of egg and nauplii production parameters of a single stock of domesticated *Penaeus monodon* (giant tiger shrimp) across generations. *Aquaculture*, 400-401, 125-128.
- Dall, W.; Hill, B.J.; Rothlisberg, P.C., and Sharples, D.J., 1990. The Biology of Penaeidae. In: Blaxter, J.H.S. and Southward, A.J. (eds.). *Advances in Marine Biology*, United States, Academic Press, 27, pp. 1-489.
- Debnath, P.; Karim, M., and Belton, B., 2014. Comparative study of the reproductive performance and White Spot Syndrome Virus (WSSV) status of black tiger shrimp (*Penaeus monodon*) collected from the Bay of Bengal. *Aquaculture*, 424-425, 71-77.
- Debnath, P.; Khan, S.H.; Karim M.; Belto, N.B.; Mohan, C.V., and Phillips, M., 2015. Review of the history, status and prospects of the black tiger shrimp (*Penaeus monodon*) hatchery sector in Bangladesh. *Reviews in Aquaculture*, 8 (3), 301-313.
- Diwan, A.D.; Harke, S.N., and Panache, A., 2018. A review on genome mapping of penaeid shrimps of commercial importance. *Aquaculture Research*, 49 (8), 2609-2624.
- Emmerson, W.D., 1980. Induced maturation of prawn *Penaeus indicus*. *Marine Ecology Progress Series* 2, 121-131.
- FAO, 2007. *Improving Penaeus monodon hatchery practices: Manual based on experience in India*, Rome: Food and Agricultural Organization of United Nations Fisheries Technical Paper. No. 446, 101 p.
- FAO, 2018. *The State of World Fisheries and Aquaculture 2018 -Meeting the Sustainable Development Goals*. Rome: Food and Agricultural Organization of United Nations, 210 p.
- Feijó, R.G.; Braga, A.; Lanes, C.F.C.; Figueiredo, M.A.; Romano, L.A.; Klosterhoff, M.C.; Nery, L.E.M.; Maggioni, R.; Wasielesky, W., and Marins, L.F., 2016. Silencing of gonad-inhibiting hormone transcripts in *Litopenaeus vannamei* females by use of the RNA interference technology. *Marine Biotechnology*, 18, 117-123.
- Gopalakrishnan, A.; Rajkumar, M.; Rahman M.M.; Sun, J.; Antony P.J.; Venmathimaran, B.A., and Trilles, J.P., 2014. Length-weight relationship and condition factor of wild, grow-out and 'loose-shell affected' giant tiger shrimp, *Penaeus monodon* (Fabricius, 1798) (Decapoda: Penaeidae). *Journal of Applied Ichthyology*, 30, 251-253.
- Harison, K.E., 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *Journal of Shellfish Research*, 9, 1-28.
- Kannan, D.; Thirunavukkarasu, P.; Jagadeesan, K.; Shetty, N., and Kumar, A., 2015. Procedure for maturation and spawning of imported shrimp *Litopenaeus vannamei* in commercial hatchery, south east coast of India. *Fisheries Aquaculture Journal*, 6, 14-16.
- Makinouchi, S. and Primavera, M.H., 1987. Maturation and spawning of *Penaeus indicus* using different ablation methods. *Aquaculture*, 62 (1), 73-81.
- Merlin, J.; Mohanlal, D.L.; Balasubramanian, C.P.; Sherly, T.; Subramoniam, T.; Syamadayal, J.; Ravichandran, P.; Ponniah, A.G.; Gopal, C., and Vijayan, K.K., 2015. Induction of vitellogenesis and reproductive maturation in tiger shrimp, *Penaeus monodon* by 17 $\beta$ -estradiol and 17 $\alpha$ -hydroxyprogesterone: *in vivo* and *in vitro* studies. *Invertebrate Reproduction Development*, 59, 166-175.
- Mohamed, S.K. and Diwan A.D., 1991. Neuroendocrine regulation of ovarian maturation in the Indian white prawn *Penaeus indicus* H. Milne Edwards. *Aquaculture*, 98 (4), 381-393.
- Mosha, E.J. and Gallardo, W., 2013. Distribution and size composition of penaeid prawns, *Penaeus monodon* and *Penaeus indicus* in Saadan estuarine area, Tanzania. *Ocean & Coastal Management*, 82, 51-63.
- Muthu, M.S., 1980. Development and culture of penaeid larvae - A review. In: Subramaniam, T. and Varadarajan, S. (eds.) *Progress in Invertebrate Reproduction and Aquaculture*, pp. 203-226.
- Muthu, M.S. and Laxminarayana, A., 1982. Induced breeding of the Indian white prawn *Penaeus Indicus*. In: Silas, E. G.; Rao, P.V.; Nair V.R., and Alagarwami, K. (eds.), *Proceedings of the Symposium on Coastal Aquaculture*, Part 1, Marine Biological Association of India, Symposium series 6, pp. 16-27.
- Muthu, M.S., Pillai N.N., and George, K.V., 1978. Larval development: *Penaeus indicus* H. Milne Edward. In: Silas, E.G. and Kartha, K.K.N. (eds.), *Larval Development*

- of Indian Penaeid Prawns: Coastal Aquaculture Marine Prawn Culture, Cochin: CMFRI Technical bulletin no: 28, pp. 12-21.
- Otoshi, C.A.; Arce, S.; Steve, M., and Moss, M., 2003. Growth and reproductive performance of broodstock shrimp reared in a biosecure recirculating aquaculture system versus a flow-through pond. *Aquacultural Engineering*, 29 (3), 93-107.
- Palacios, E.; Ibarra, A.M., and Racotta, I.S., 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. *Aquaculture*, 185, 353-371.
- Palacios, E. and Racotta, I.S., 2003. Effect of a number of spawns on the resulting spawn quality of 1-year old pond-reared *Penaeus vannamei* (Boone) brood stock. *Aquaculture Research*, 34, 427-435.
- Palacios, E.; Racotta, I.S.; Heras, H.; Marty, Y.; Moal, J., and Samain, J.F., 2001. Relation between lipid and fatty acid composition of eggs and larval survival in white pacific shrimp (*Penaeus vannamei*, Boone, 1931). *Aquaculture International*, 9, 531-543.
- Peixoto, S.; Cavalli, R.O., and Wasielesky, W., 2005. Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis*. *Brazilian Archives of Biology and Technology*, 48 (6), 997-1006.
- Prasad, G., 2001. Length-weight relationships of *Penaeus monodon* reared in semi-intensive culture systems of Kerala, India. *Naga, ICLARM Q.* 24, pp. 16-17.
- Primavera, J.H., 1985. A review of maturation and reproduction in closed thelycum penaeids. In: Taki, Y.; Primavera, J.H., and Llobrera, J.A. (Eds.), *Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps*, (Iloilo City, Philippines), pp. 47-64.
- Tomy, S.; Saikrithi, P., Nithoon, J.; Balasubramanian, C.P.; Panigrahi, A.; Otta, S.K.; Subramoniam, T., and Ponniah, A.G., 2016. Serotonin-induced changes in the expression of ovarian gene network in the Indian white shrimp, *Penaeus indicus*. *Aquaculture*, 452, 239-246.
- Vijayan, K.K., 1989. Studies on the physiology of moulting in the penaeid prawn *Penaeus indicus*, H. Milne Edwards. Kerala, India: Cochin University of Science and Technology, Ph.D. dissertation, 265 p.
- Vijayan, K.K., 2019. Towards domestication and genetic improvement of native Indian white shrimp, *Penaeus indicus*: a complimentary desi option to exotic *Penaeus vannamei*. In: *Book of Abstracts World Brackishwater Aquaculture Conference (BRAQCON 2019)*, ICAR-Central Institute of Brackishwater Aquaculture (CIBA) & Society of Coastal Aquaculture and Fisheries (SCAFi), Chennai, India. p. 40.
- Wyban, J., 2007. Domestication of Pacific white shrimp revolutionizes aquaculture. *Global Aquaculture Advocate*, 42-44.