Enhancing the Reproductive Performance of Cultured Shrimp: novel information on scent, maturation, and mating

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What are sex pheromones and how do they function in the courtship and mating of shrimps and other crustaceans? Best known as chemical compounds excreted by animals to initiate reproduction-related responses from their potential mates, pheromones became an important factor in shrimp culture. An earlier study, showed that the domesticated shrimp had a reduced level of pursuit behavior than the wild-caught and suggested that sex pheromones could have played a role in this behavioral difference. Domesticated penaeids are typically reared communally until ready for use as broodstock wherein animals are continually exposed to varying chemical cues. There is a close relationship between chemicals involved in molting and mating of closed thelycum species but information regarding the effect of prolonged exposure of male penaeids to molting and sex pheromones of females on reproduction behavior is not available.

In 2021, SEAFDEC/AQD conducted a research to evaluate the possible implications of monosex rearing on gonad maturation and reproduction behavior of *Penaeus indicus* broodstock. Results demonstrated that the traditional communal rearing of male and female *P. indicus* broodstock do not have a significant effect on the ovary development and sperm quality of female and male broodstock, respectively. But this common practice of rearing male and female broodstock together significantly reduced reproductive related behavior leading to lower successful matings compared to when broodstock were reared monosex. This novel information could be linked to previous report on reduced successful matings previously reported in black tiger prawn, *P. monodon*. Therefore, monosex rearing should be considered as an alternate broodstock setup as this will facilitate optimized sex-specific nutritional manipulation and even potentially increase the mating success of domesticated penaeid broodstock.

Shrimp is the most traded aquaculture commodity and is considered a significant contributor to world aquaculture production. The shrimp industry is projected to expand another 50 % in its production; however, disease outbreaks continue to threaten the industry across the globe. Different approaches were carried out to address this problem, including closing the life cycle in captivity and domesticating the commodity.

However, wild-caught broodstock is a known vector of pathogens and using wild animals in the hatchery phase was reported as the culprit of introducing pathogens to production systems. Addressing this issue would not only prevent the risk of pathogen introduction from wild-caught broodstock but also ensure a steady supply of high-quality larvae. Most importantly, the foundation of high-quality domesticated broodstock is a prerequisite for developing pathogen-free broodstock which will lead to genetically better culture lines in the future.

Domestication of some penaeid species resulted in poor broodstock reproductive performance as compared to wild-caught. Females gathered from the wild matured and reproduced more quickly and frequently than domesticated females after ablation (Coman *et al.*, 2006). Subsequently, wild-caught females were also said to produce more eggs than domesticated broodstock (Menasveta *et al.*, 1993; Arnold *et al.*, 2013). Furthermore, the domestication of black tiger prawn (*Penaeus monodon*) resulted in poorer reproductive performance than that of the wild-caught due to relatively low mating success in domesticated broodstock. An earlier study showed that the domesticated shrimp had a reduced level of pursuit behavior than the wild-caught and suggested that sex pheromones could have played a role in this behavioral difference (Marsden *et al.*, 2013).

A breeding program would usually start by collecting wild species of interest, growing them in tank systems, closing the life cycle in captivity, and domesticating several generations to develop culture lines with superior growth, improved disease resistance, and prolific reproduction. The black tiger prawn used to dominate global shrimp production; however, disease outbreaks and difficulty in domestication prompted the shift to alternate species.

**Sex pheromones, the shrimp scent for mating**

Pheromones are chemical messages that an individual secretes to elicit a behavioral reaction or an endocrine change in another individual of the same species (Altstein, 2013). In addition, sex pheromones are chemical compounds released by animals to communicate many critical life-cycle processes, such as locating conspecifics, recognizing another individual as potential mates, and orienting various body parts for effective sperm transfer during reproduction (Caskey and Bauer, 2005; Snell *et al.*, 1995). In short, sex pheromones are like scents that are released by the females to advertise its readiness to mate, its location, and its body orientation.

These chemical cues were reported to have evolved from substances leaked from sexually mature adults or released along with eggs or sperm stored in the body cavity. The exchange of small quantities of egg and sperm coupled with its respective chemical cues leads the female to release her eggs together with a large amount of pheromones to prompt the male to release a substantial amount of sperm into the
eggs (Wyatt, 2014). Pheromones are generally identified as proteins and peptides which co-opted from molecules with other functions. The blend of these chemicals changes along with the divergence or convergence of species, such that related species would differ by just two amino acids (Rittschof & Cohen, 2004).

To better understand what causes the poor mating success in the domesticated *P. monodon*, the study of Marsden et al., (2013) clarified whether differences exist in mating behavior and hence mating success between wild-caught and domesticated *P. monodon* broodstock. Matings were observed only involving pairings where the female originated from the wild and not when females were domesticated, and molted females were pursued more vigorously by wild line males than domesticated line males. Specifically, wild line males spent more time “under” newly molted females than domesticated line males. Likewise, wild line females more often had males “under” them than domesticated line females. This reduced engagement in courtship and mating rituals which led to reduced mate recognition could create a big impact on the overall reproductive success of *P. monodon* in hatcheries. It was speculated that the shrimp’s reduced level of pursuit behavior was probably due to the smaller amount of ineffective pheromones produced by the domesticated female shrimp. It may also be that the domesticated males have a reduced ability to detect or process the chemicals compared to their wild-caught counterparts.

**Nutritional requirements of male and female Penaeid broodstock**

Nutritional manipulation is one of the most sought interventions to improve the reproductive performance of domesticated broodstock. Researchers examined the effects of different food formulations and diet combinations on vitellogenesis and vitellogenin levels, biochemical composition, spawning success, and fecundity. However, there is a lack of study on the effects of these dietary interventions on male shrimp species. Maturation diets that were optimized to promote female maturation were the same feed provided for male broodstock. This is despite reports on some crustaceans showing variances in the nutrient requirements of males and females for growth. Similarly, the kind and amount of nutrients required by male and female broodstock may also vary since each broodstock undergoes different physiological and behavioral processes to successfully reproduce.

Female shrimp invests energy in the accumulation of yolk protein during vitellogenesis, which is subsequently utilized during embryogenesis and early larval development of the offspring. For instance, a fully mature ovary of a female penaeid such as Indian white prawn *P. indicus* weighs up to 6.00 % of the total body weight. Meanwhile, for males, reports showed that mature spermatophore could only weigh as much as 0.12 % of body weight based on a 20–30 g *P. stylirostris* (Alfaro, 1993) and up to 0.64 % of body weight for *P. indicus* (Santander-Avanceña, 2021). For males, reproductive energy investments were spent on reproductive-related behavior such as mate searching and courting, which were guided by different cues. These variances in the gonadosomatic indices and demand for energy during mating between male and female penaeids could have implications in the respective nutritional requirements of male and female broodstock.

For efficient shrimp broodstock maturation, polychaete is an indispensable diet. But what is in the polychaete that specifically promotes ovarian development and sperm production? Santander-Avanceña et al. (in press) tested the most efficient polychaete component in promoting female and male penaeid maturation. Mud polychaete (*Marphysa* sp.) was used in the research and the extract was fractionated into a total soluble fraction (TSF), neutral lipid fraction (NLF), and polar lipid fraction (PLF). The study was conducted on the Indian white prawn, a commercially important closed thelycum penaeid species indigenous to the Philippines. The broodstock, *P. indicus* was fed with an artificial maturation diet with polychaete extract fraction supplemented at different inclusion levels (0.25 %, 0.50 %, and 1.00 %) following a 3 × 3 factorial design. The study was conducted using 500-L tanks, and a total of 33 units of tanks were used for females and another set of 33 tanks for the male study (Figure 1).

It is widely known that polychaete is regarded as the best maturation diet because of its high content of highly unsaturated fatty acids and reproductive hormones. Results confirmed the efficiency of NLF to enhance gonad maturation with at least a 0.50 % inclusion level. It was also proven that other fractions such as TSF containing protein and peptide precipitates and PLF containing phospholipids can equally promote maturation (Figure 2). This study also demonstrated the efficiency of supplementing polychaete phospholipids in promoting vitellogenesis of *P. indicus*. This fraction promoted...
the gonad maturation in the greatest number of female broodstock and the highest relative expression vitellogenin ovarian gene at the lowest inclusion level (0.25 %). Real-time PCR analysis of vg transcripts in ovaries of *P. indicus* broodstock showed similar levels of gene expression for all treatments (1.02–1.93) except for the group fed with a maturation diet supplemented with 0.25 % and 0.50 % polar lipid fraction where vg expression is approximately four-fold higher. However, this maturation promotion effect of PLF was not consistent with the male *P. indicus* broodstock since PLF extract supplementation resulted in lower sperm counts of broodstock compared to that fed supplemented with TSF and NLF.

It is also noteworthy that the provision of a well-formulated diet without polychaete supplementation resulted in similar maturation rate sperm quality, highlighting the differences in the nutritional demands of male and female broodstock during maturation. Such variation in the nutritional requirement in terms of growth of male and female were also reported for other crustaceans such as those for *P. monodon* (Hansford & Hewitt, 1994) and *Eriocheir sinensis* (Zhu et al., 2021). Hence, sex-specific nutritional manipulation can be performed to address the precise needs of male and female broodstock.

**Ovarian maturation and sperm development of communally reared (conditioned) and separately reared (naive) male and female broodstock were monitored for one month (Figure 3).** Conditioned broodstock are the broodstock from the communally reared group, while naive broodstock refers to the test animals grown separately after secondary sexual identifications were visible. The broodstock used was the same batch of post-larvae divided into different treatment groups after secondary sexual organs were visible. The conditioned broodstock was previously exposed to molting hormones and sex pheromones released by counterparts since stocking in tanks.

Based on daily monitoring of gonad development, results showed a higher percentage of female broodstock reared with males developing mature ovaries, but the difference was insignificant. Similarly, it only took seven days for communally raised females to develop mature gonads compared to those reared in all-female tanks, around 11 days, but the difference was not significant. A previous report on caridean shrimp (*Neocaridina davidi*) showed male presence did not enhance the percentage of mature females in caridean shrimp (Tropea et al., 2018), but it successfully boosted ovary sizes. The maturation rate and latency duration were likewise higher in conditioned females in the current investigation, but the stimulatory effect of pheromones was not significant.

This finding suggests that other factors have far more significant impacts on vitellogenesis than the stimulus provided by male counterparts. Eyestalk ablation was performed on female broodstock from both treatments. In the process of removing the eyestalk, vitellogenesis-inhibiting hormone was removed, and the release of gonad-stimulating factor was stimulated (Wongsawang et al., 2005). Furthermore, the nutritional levels in both treatments’ diets were previously suited for *P. indicus* maturation, which could have helped gonad maturation (Santander-Avanceña et al., 2020).

Regarding males, growing the broodstock either in monosex tanks or communally with females did not significantly impact the percentage of males developing mature sperm nor affected the interspermatophore period or the number of days elapsed for the broodstock to develop mature sperm. The quality of sperm was also not affected by the different rearing methods,
specifically, the sperm counts, percent viability, and percent abnormality values were similar between the two groups. Currently, there is no data available on the influence of female presence on male penaeid maturation. Similarly, the presence of females did not influence the sperm production of rainbow trout, *Salmo gairdneri* (Büyükhatipoglu & Holtz, 1984). According to research, male guppy *Poecilia reticulata* coupled with a female for one week had more strippable sperm (Bozynski & Liley, 2003). Male *Macrobrachium rosenbergii* prawns cocultured with late premolt to early postmolt females had significantly increased gonad maturation (Kruangkum *et al.*, 2019). Sperm decrease was also observed in other creatures due to the absence of females or reduced exposure to females (Bjork *et al.*, 2007; Demas & Nelson, 1998; Wedell *et al.*, 2006). The sperm count and quality recorded in this study were within the range of sperm counts previously published for penaeid species, implying that sperm development did not require exposure to females if the nutritional requirements of the male broodstock were met.

Mating behavioral differences between naive and conditioned broodstock were also compared using the same broodstock set. Daily monitoring was done to identify premolt females. Penaeid shrimps, such as *P. indicus*, are closed thylecum species and can only mate if it is in the molt stage. During that stage, sex pheromones are released by females to signal the male counterpart the readiness to mate. The identified premolt females were transferred to 100-L glass aquaria and paired with two intermolt males or hardshell broodstock. Each aquarium was equipped with cameras for overnight video surveillance. Red lighting was also provided to improve visibility but will not disturb the activities of the animals (Figure 4).

Reproductive behaviors were assessed based on the total counts of the two males touching discarded molt (touch), fighting with another male (fight), chasing the female (chase), and advancing to a probing position (probe) where the male was swimming under the female (Marsden *et al.*, 2013). The number of times a male fought with another male was also counted. The sum of all reproduction-related behaviors was compared for the two treatments. Activities were noted and analyzed within 30 min of molting (Marsden *et al.*, 2013). Some reproductive behaviors assessed are shown in Figure 5.

![Figure 4](image1.png)

*Figure 4. Reproductive-related behavior and mating of naive and conditioned *P. indicus* broodstock were observed in a 100-L glass aquarium for overnight video surveillance equipped with red lights and cameras.*

![Figure 5](image2.png)

*Figure 5. Reproductive-related behavior of *P. indicus* broodstock towards successful mating (a: male broodstock chased a newly molted female; b: advanced to probing position; c: a male curved its body perpendicular to female and flexed its head and tail simultaneously to deposit sperm).*

Results showed that males from the two groups had statistically significant differences in initiated chase and fight, with the naive males recording more chase and fight counts than the conditioned males. Naive males were also more likely to advance to the probing position and touch a discarded molt than conditioned males, although the differences were not significant (Figure 6). No significant difference was detected in the counts of probe and touch between naive and conditioned males but significant differences in the occurrences of chase, fight, and sum were determined.
The analysis of the sum of total reproductive-related behavior showed that naive males initiated considerably more activities (54.00) than conditioned males (24.20) (Figure 7). Animals generate sex pheromones to convey numerous crucial life-cycle events. Detection of this chemical cue is vital for closed thylecum species to secure a mating partner during the limited time the female is in the molt stage. Males that were raised away from females exhibited higher reproductive behavior than conditioned males. Constant exposure to molting females and their accompanying sex pheromones might have affected the male identification of molting females. However, no data on the effect of continual exposure on penaeid reproductive behavior is available.

Nonetheless, insects have been observed to have different sensitivities to sex pheromones. Pre-exposure to sex pheromones in nuisance insects like Copitasia decolora and Grapholita molesta inhibited the olfactory response, lowering attractiveness during mate finding, and delaying mating (Figueroado and Baker, 1992; Robledo et al., 2018). While this condition is used to manage pests, it will have a negative impact on the reproductive performance of economically significant penaeids like P. indicus. Furthermore, the initial results on the effect of the rearing strategy on mating revealed a 60% difference in successful mating between naive and conditioned.

According to Okumura (2004), there is no clear connection between vitellogenesis and the molt cycle. However, because molting is required for closed thylecum female species like P. indicus and P. monodon to mate (Primavera, 1985), the emission of sex pheromones may occur during premolt stage of domesticated shrimp, especially when ecdysteroid levels are at their highest (Okumura, 2004). The desensitization to sex pheromones noted in insects could be the same condition that happened in the conditioned P. indicus broodstock. The reduced initiation of reproductive related behavior in conditioned male broodstock group which lead to low successful mating could be due to prolonged exposure to mate inducing scents or sex pheromones. While the concept of desensitization is utilize in agriculture to control the population of pests, this could have a negative implication in shrimp aquaculture.

Conclusion and Way Forward

This is the first study to report the effects of extended exposure of male penaeids to female molting and sex pheromones on reproductive behavior. The study also showed that the traditional practice in hatcheries of communal rearing male and female penaeids did not negatively impact gonad maturation but may still affect overall reproductive output by reducing the broodstock reproductive behavior. This novel information can be possibly linked to the reduced reproductive performance of captive penaeids compared to the wild-caught which is a bottleneck in domesticating the major aquaculture commodity, P. monodon.

The use of polychaete extract as a supplement stimulated the development of new tactics for nutritional manipulation of male and female penaeid broodstock. To optimize maturation and mating, communal stocking of male and female broodstock until suitable for use should be re-evaluated. The present study on the effects of communal versus individual broodstock rearing on maturity and reproductive behavior demonstrated that growing monosex or co-cultured broodstock did not affect maturation and sperm production and quality of shrimps – it can even improve reproductive behavior and mating success. These findings point to the possibility of a nutritional intervention tailored for male and female broodstock.

As a former major producer of black tiger prawn and since it is indigenous to the Philippines, it would be of great interest for the country and valuable for the scientific community to know if indeed the failing pheromones, low production and failure in detection, played a key role in the reduced reproductive behavior.
output of domesticated stocks of the species. Answering this knowledge gap could be the linked to regain the glorious days of the Philippine prawn industry.

Isolation and identification of sex pheromones used by crustacean is a challenging research endeavor as suggested by the limited scientific output in this line of work. However, pheromones specifically sex pheromones play a significant role in an organism's successful reproductive performance and possible application in aquaculture especially in breeding will be a big lift to the industry. To be able to determine receptive females whose mating and spawning are fairly synchronized would be useful for hatchery operations. This would allow holding synchronized females together that would populate the spawning tank with postlarvae which are uniform in age and size.

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References

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