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A STUDY OF FACTORS INFLUENCING THE HATCH RATE OF *PENAEUS VANNAMEI* EGGS. II. PRESENCE OF A SPERMATOPHORE

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ABSTRACT Eighty-three mated *Penaeus vannamei* females were sourced from a commercial sized maturation tank. The hatch rate was recorded for those shrimp based on the presence of a full spermatophore, a partial spermatophore or the loss of the spermatophore during sourcing and handling. The hatch rates were not significantly different among females for the three spermatophore conditions. The mean hatch rates were 48.8% for full spermatophores, 43.1% for partial spermatophores and 55.6% for lost spermatophores. The location of the sperm at fertilization and the precise mechanisms of fertilization are still unknown.

INTRODUCTION

Reproduction of open thelycum penaeid (Litopenaeidae) shrimp has been discussed by Chamberlain (1985), Dall et al. (1990), Bray and Lawrence (1992), and Browdy (1992). Ovarian maturation in open thelycum penaeid shrimp occurs during the intermolt cycle of the adult female. Mating takes place soon after dusk, four to five hours prior to spawning. Mating is accomplished with the males' transference of a compound spermatophore to the female's thelycum. Apparently, fertilization of the eggs occurs simultaneously with spawning.

Early researchers working with *P. setiferus* were rarely able to collect females with attached spermatophores (Andrews 1911; Burkenroad 1934; Heegaard 1953). Early reports noted that the spermatophores of *P. setiferus* are easily dislodged (Weymouth et al. 1933; King 1948; Cook and Murphy 1966; Perez-Farfante 1969, 1975). In fact, Weymouth et al. (1933) reported that out of 18,487 females examined, spermatophores were found on only 20 of the animals. Cook (1967) obtained fertilized eggs from female *P. setiferus* bearing no spermatophores. While examining wild female *P. setiferus* in which no spermatophores were found attached, Bray et al. (1983) detected minute sperm masses 2 mm in diameter. These sperm masses cannot be seen unless the third pair of walking legs are folded back and the thelycum closely examined. Of 103 mated animals examined from the wild, they noted the condition of a "sperm mass only" to be most prevalent (54%), as opposed to partial spermatophores (19%) and full spermatophores (27%). They also noted the sperm mass only condition for *P. setiferus* held in tanks. There was no significant difference in the number of nauplii or the hatch rate for the three spermatophore conditions.

Bueno (1990), working in tanks with another open thelycum Litopenaeid, *P. schmitti*, noted 79% of the females with full spermatophores and 21% of the mated females with sperm mass only. He found no significant effect when correlating the spermatophore condition with fertilization and hatch rate.

During the captive reproduction of *P. vannamei*, mature mated females are removed from the large maturation tanks and placed in small spawning tanks. The females are selected based on the presence of either a full or partial spermatophore. It is also common for the full spermatophores of *P. vannamei* to become dislodged and lost during handling. In an attempt to document the effect of the spermatophore condition on hatch rates for *P. vannamei*, the following data are presented.

MATERIALS AND METHODS

The shrimp, *P. vannamei*, were matured and mated in large commercial sized maturation tanks (Ogle 1992). Mated females were sourced for mating and removed from the maturation tanks in the evening. Mated females were placed, one per tank, into 1m² round fiberglass spawning tanks containing 100 L of seawater (Ogle 1995). Prior to sourcing, the spawning tanks were filled with filtered baywater which had been adjusted from 25 ppt to 30 ppt salinity by the addition of an artificial seasalt (Marine Environment, San Francisco, CA). Moderate aeration was provided by a single airstone. The shrimp were checked for spawning after two to three hours and spent females were returned to the maturation tank.

The number of eggs was estimated by subsampling. The water in the spawning tanks was stirred and five 10 ml

subsamples were collected from the four compass directions and from the tank center. The samples were transferred to a petri dish and the eggs counted. Data were averaged and the total number of eggs calculated. After 12-15 hours, the number of nauplii was determined in the same fashion and the hatch rate calculated.

A total of 83 mated females was sourced from the maturation tanks. Condition of the spermatophore (full, partial or lost during sourcing) was noted. The effect of the three spermatophore conditions on the hatch rates of all spawns was compared by AVOVA where alpha < 0.05 was significant. In some of the individual spawns, none of the eggs hatched. The spawns which produced no nauplii (no hatch) were eliminated from the data set and the data reanalyzed.

RESULTS

The hatch rate of *P. vannamei* eggs was not significantly influenced by the loss or partial presence of the spermatophore (Table 1). The hatch rate for 49 shrimp retaining a full spermatophore was 31.8% (S.E. 4.68). The hatch rate for the 16 shrimp retaining only a partial spermatophore was 24.2% (S.E. 7.52). The hatch rate for the 18 shrimp which lost their spermatophores was 18.2% (S.E. 5.56). These differences were not significant given the large range in hatch rates (0-100%) and correspondingly large standard error.

When the spawns which did not hatch were eliminated from the data set, there was still no significant effect of the

spermatophore condition on hatch rate. The hatch rate for 32 shrimp with a full spermatophore was 48.8% (S.E. 5.03). The hatch rate for nine shrimp with a partial spermatophore was 43.1% (S.E. 9.33). The hatch rate for ten shrimp with no spermatophore was 32.7% (S.E. 7.24). These differences are not significant given the range in hatch rates (3.8-100%) and correspondingly large standard error.

DISCUSSION

There is no significant effect of the spermatophore presence at the time of spawning on hatch rate of *P. vannamei*. This was the conclusion reached by Bray et al. (1983) for *P. setiferus*. The 13 shrimp with full spermatophores produced 53,000 (S.E.M. ± 24,700) nauplii for a hatch rate of 26.2% (S.E.M. ± 9.13). The 11 animals with wings only produced 109,000 (S.E.M. ± 38,400) nauplii for a hatch rate of 37.2% (S.E.M. ± 8.79). The 52 animals retaining a sperm mass only produced 92,000 (S.E.M. ± 13,800) nauplii for a hatch rate of 35.2% (S.E.M. ± 3.84).

Bueno (1990), working with *P. schmitti*, could find no significant effect on either hatch rate or fertilization rate due to the condition of the spermatophore. He reported that 408 shrimp with full spermatophores produced an average of 76,558 nauplii (s.d. ± 42,694) per shrimp and 110 shrimp with a partial spermatophore produced an average of 89,903 (s.d. ± 54,386) nauplii per shrimp. In addition, he also examined the eggs and calculated a percent

TABLE 1
Hatch rate of *Penaeus vannamei* in relation to spermatophore condition.

	Full	Partial	Lost
All spawns			
% hatch	31.8	24.2	18.2
n	49	16	18
max	100.0	100.0	63.4
min	0.0	0.0	0.0
SE	4.68	7.52	5.56
Spawns that hatched			
% hatch	48.8	43.1	32.7
n	32	9	10
max	100.0	100.0	63.4
min	3.8	8.0	6.4
SE	5.03	9.33	7.24

fertilization. Not all fertilized eggs hatch. For the shrimp with full spermatophores, the percent fertilization was 73.46 (s.d. \pm 28.03). For the shrimp with a partial spermatophore, the percent fertilization was 71.50 (s.d. \pm 30.78). Despite the large sample size, significant differences could not be determined due to the large variations that exist in fertilization and hatch rates for marine shrimp.

Weymouth et al. (1933) reasoned that since the spermatophores are easily dislodged from the females, the eggs must be spawned and fertilized before the spermatophores are lost. Although we now know this is not the case, the actual mechanisms behind egg fertilization in Litopenaeid shrimp is still unclear.

Mated females are sourced out of maturation tanks 1-2 hours after mating has taken place. It has been suggested that the spermatophore ruptures (Perez-Farfante 1975; Bente 1977) and that sperm present on the pereopods of the female (Heldt 1938; Hudinaga 1942) fertilize the eggs as they brush past. To date, efforts at this laboratory to microscopically verify the presence of sperm on the pleopods and pereopods of spawning *P. vannamei* have been unsuccessful. The artificial placement of a sperm mass at several locations on mature *P. setiferus* did not significantly affect the hatch rates (Bray et al. 1983), although the hatch rate of the artificially inseminated shrimp was significantly less than that of naturally mated shrimp.

It is not known how sperm are released from the spermatophore. Spermatophores placed in test tubes of seawater at this laboratory did not rupture or release sperm even after five hours exposure. In some cases when *P. vannamei* are entirely quiescent during spawning, the eggs descend without coming into contact with the spermatophore, pleopods or pereopods, but they still hatch (Ogle, personal observation). In such cases, a

dense mass of eggs are deposited on the bottom of the tank and the hatch rate is extremely low.

King (1948) stated that the spermatophore opened and released sperm at the time of spawning, which in turn may be caused by a substance secreted with the expelled eggs. King felt that this substance may chemically or physically break down the spermatophore. In contrast, as verified in this report, fertilization of the eggs is accomplished even though the spermatophore is completely lost prior to spawning. Therefore, it is suggested here that the sperm or sperm mass is released from the spermatophore shortly after mating and several hours before spawning. The location of the sperm at the time of fertilization and the mechanism of egg fertilization are unclear, as is the "need" for the rather complex spermatophore. Female shrimp have been observed manipulating the spermatophore with the pereopods after mating (Ogle, personal observation). It is not known whether this ruptures the spermatophore or possibly transfers sperm to the oviducts.

This report substantiates for *P. vannamei*, as for *P. setiferus* and *P. schmitti*, that the presence of the spermatophore at spawning does not significantly affect the hatch rate.

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