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### A STUDY OF FACTORS INFLUENCING THE HATCH RATE OF *PENAEUS VANNAMEI* EGGS. I. EFFECTS OF SIZE, SHAPE AND VOLUME OF THE SPAWNING TANK

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ABSTRACT The hatch rate of *Penaeus vannamei* eggs spawned by individual females in square and round tanks and in different volumes of seawater was determined. The mean hatch rates ranged from 17.3% to 64.9% and were not significantly different for volumes of 50, 100 and 200 L nor for square or round tanks of equal water volumes. Hatch rate was significantly affected by the size of the spawning tank.

#### INTRODUCTION

Aquaculture accounts for over 25% of the world's shrimp production (Rosenberry, 1991). The farming of shrimp requires acquisition of seed stock which is reared to marketable size. Seedstock may be harvested from the wild or produced from broodstock. Broodstock may be sourced from the wild or acquired from maturation facilities. In either case, mated oviparous shrimp are spawned under captive conditions in order to collect the larvae. A variety of systems have been used to spawn marine shrimp. Eggs or nauplii may be collected directly from the tank in which maturation and matings take place (Laubier-Bonichon and Laubier 1979; Brown et al. 1980; Lawrence et al. 1980; Simon 1982; Chen et al. 1991; Ogle 1992). However, many facilities remove the mated females to a separate tank for spawning. Wyban and Sweeney (1991) and Bray and Lawrence (1992) report the stocking of a single mated female into a tank while Lawrence et al. (1980), Kittaka (1981), Aquacop (1983), Yang (1975), Tabb et al. (1975) and Treece (1985) cite that several mated females may be placed in a common tank for spawning. In most cases, spawning and hatching are accomplished in one tank and the nauplii are collected and transferred to another tank for larval rearing. However, Salser (1978) and Bray and Lawrence (1992) recommend that after the shrimp spawn, eggs be removed to a separate tank for hatching. Tanks utilized for spawning and hatching have been as large as 145,800 L (Kittaka, 1981) and as small as 10 L (Mock and Murphy 1971). Bray and Lawrence (1992) suggest 75 L as the minimum tank size with 100 to 150 L circular tanks supplied with lids as the preferred choice for spawning and hatching. Browdy (1992) suggests using a tank size of 150-500 L and Browdy and Samocha (1985a and 1985b) report that simple flat-bottom tubs are sufficient for the spawning and hatching of eggs. Aquacop (1983) utilizes conical bottom tanks. A comparison of the two tank types, conical and flat-bottomed, with *P. setiferus* could demonstrate no significant differences in hatch rates (Browdy, in preparation). Lotz and Ogle (1994) report an increasing hatch rate of *P. vannamei* with an increasing volume of the spawning tanks. However, the spawning tanks varied in size and shape. As these are the only comparative studies on the effect that tank shape and volume have on hatch rates, the present study was undertaken with *P. vannamei*.

#### MATERIAL AND METHODS

Three different tank types were used for spawning mated *P*. vannamei females. Rectangular polyethylene tubs, 0.51 m x 0.53 m x 0.32 m, with a bottom area of  $0.27 \text{ m}^2$  were utilized as one of the tank types. The other two tank types were both round fiberglass tanks and are referred to as small and large. The small tanks had a diameter of 0.61 m and a depth of 0.45 m with a bottom area of  $0.29 \text{ m}^2$  while the large tanks had a diameter of 1.12 m and a depth of 0.60 m with a bottom area of  $0.98 \text{ m}^2$  (1 m<sup>2</sup>).

The spawning tanks were filled with a measured volume of seawater. Natural baywater pumped from Davis Bayou in the Mississippi Sound was allowed to stand for several weeks to allow suspended solids to settle. Artificial seasalt (Marine Environment, San Francisco, CA) was added to increase the ambient salinity of 25 ppt to a salinity of 30 ppt. The water was filtered through a particulate five-micron RS pleated polyester fabric media cartridge (Amtek, Flowrite, Inc., 3345 Halls Mill Rd., Mobile, AL 36606) and granular activated carbon (Amtek) before use. Sodium EDTA was added at the rate of 3 ppm.

Shrimp were matured and mated in large commercial sized maturation tanks (Ogle 1992). Mated females were sourced from the maturation tanks in the evening and placed individually into the spawning tanks. Moderate aeration was provided by a single airstone throughout spawning and hatching. The shrimp were checked for spawning after two to three hours and spent females were returned to the maturation tanks.

The number of eggs was estimated by subsampling. The water in the spawning tank was stirred and five-10 ml subsamples were collected. Subsamples were taken from the four compass directions and the center of the tank. 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 (% H = # nauplii/# eggs x 100). Only spawns that hatched were analyzed. Data were compared by ANOVA and significant (alpha = 0.05) differences noted.

The large  $(1m^2)$  tanks were used to study the effect of water volume on hatch rate with 50, 100 or 200 L of seawater placed in a tank for each shrimp. Twelve shrimp were individually spawned in each of the three water volumes, for a total of 36 shrimp. Fifty L of seawater were used in all subsequent studies. The effect of tank shape on hatch rate was determined by comparing spawns in the square tubs, 0.27 m<sup>2</sup>, and the small fiberglass round tanks, 0.29m<sup>2</sup>. A total of 14 individual spawns were recorded for the square tanks and 15 individual spawns were recorded for the small round tanks. An additional 14 animals spawning in the small round fiberglass tanks were compared to 14 animals which spawned in the large round fiberglass tanks to determine the effect of tank size on hatch rate. In all studies, eggs were left in the spawning tanks until hatching occurred. After the eggs hatched, the tanks were drained, cleaned and filled with new seawater.

#### RESULTS

There were no significant differences for the effect of the water volume in the spawning tanks or the shape of the spawning tanks on hatch rates of *P. vannamei* eggs (Table 1). The average hatch rates for eggs spawned in 50, 100 and 200 L of water were 45.2, 55.2, and 42.4%, respectively. The average hatch rates for eggs spawned in the square and the small round tanks were 25.4 and 18.2%, respectively.

There was a significant effect of tank size on the hatch rates. The hatch rates for eggs spawned into a tank of  $0.29 \text{ m}^2$  and  $1 \text{ m}^2$  were 17.3% and 64.9%, respectively.

Overall, the minimum spawn size was 39,000 eggs and the maximum spawn size was 230,000 eggs. The minimum egg density was 780/L and the maximum was 4,600 eggs/L. This is equivalent to 10,000 to  $900,000 \text{ eggs}/m^2$ .

Study	Treatment	- Replicates N	Hatch Rate			
			Mean %	Range %	S.E.	Significance
Volume	50 L	12	45.2	3.8 - 89.3	7.10	NS
	100 L	12	55.2	15.2 - 100.0	7.21	NS
	200 L	12	42.4	1.5 - 90.0	8.54	NS
Shape	Square	14	25.4	3.8 - 57.8	4.15	NS
	Round	15	18.2	2.4 - 51.3	5.26	NS
Size	0.29 m <sup>2</sup>	14	17.3	2.0 - 51.3	4.15	SIG
	1.00 m <sup>2</sup>	14	64.9	15.2 - 100.0	6.69	SIG

#### **TABLE 1**

The effect of tank volume, shape and size on the hatch rate of *Penaeus vannamei* eggs.

Volume - 1 m<sup>2</sup> round fiberglass tank. Shape - square, 0.27 m<sup>2</sup> polyethylene tanks with 50 L of water; round, 0.29 m<sup>2</sup> fiberglass tanks with 50 L of water. Size - round tanks with 50 L of water. SIG = significant. NS = non-significant.

#### DISCUSSION

While there was a significant effect of the tank size on the hatch rate of *P. vannamei* eggs, the reason is unclear. The volume in both the large and small tanks was 50 L which created a greater water depth in the smaller tank. The difference in depth does not appear to have an effect on hatching as demonstrated in the experiment on water volumes in this study. This suggests that as long as the female is allowed a sufficient area for unrestricted spawning, the depth of the water beneath the shrimp appears to have no effect on hatch rate. Instead, tank area may play an important role in hatch rate. The average hatch rate reported for *P. vannamei* of 30% to 47% (Wyban and Sweeney 1991) is consistent with the rates reported here of 17.3% to 64.9%.

It should be pointed out that the hatch rates reported here include three discrete events: spawning, fertilization and hatching. Spawning of *P. vannamei* occurs as the female slowly swims in circles near the surface of the water. It is possible that restricting the movement of the shrimp to a smaller area may interfere with fertilization of the eggs which would result in a lower hatch rate. It is also possible that the hatch rate may be directly influenced by the bottom area of the tank.

As the mechanisms involved in egg fertilization are not known, it is unclear how restricting the swimming activity of the shrimp during spawning would influence fertilization and hatch rates. Heldt (1938) and Hudinaga (1942) have suggested that sperm released from the spermatophore are trapped by the ventral setae of the third and fourth pereopods which come into contact with the eggs as they are released. We have been unable to detect sperm on the pleopods or pereopods of mated *P. vannamei* by microscopic examination. Fertile spawns frequently result from matings for which the spermatophore is missing. Fertile spawns have also resulted from females which do not swim but lie perfectly still during spawning. In such incidence, the hatch rate is extremely low (Ogle 1993b).

The effect of egg density on hatching is also unknown. Primavera (1980) has recommended that the egg density in spawning tanks for *P. monodon* not exceed 2,500 to 3,000 eggs per liter. In another study, Primavera *et al.* (1977) found no effect on the hatching of *P. monodon* eggs at densities of 7000/I. However, they did not report the results in terms of area (eggs/m<sup>2</sup>). *P. monodon* eggs are larger than *P. vannamei* eggs (Ogle, in preparation) so the densities used here of 780-4,600 eggs/L should have no effect on the results. This is also supported by the fact that the volume of water did not significantly affect the hatch rate. Further research could be directed to determined the effect of tank area on fertilization rates and hatch rates.

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