

*Asian Fisheries Science* 6(1993):283-293.  
Asian Fisheries Society, Manila, Philippines  
<https://doi.org/10.33997/j.afs.1993.6.3.004>

## **The Effect of Substrate Type on the Ovarian Maturation of *Penaeus japonicus* Bate**

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### **Abstract**

Unilaterally eyestalk ablated female *Penaeus japonicus* produced more spawns per shrimp and more eggs in total in maturation tanks with a hard substrate than in tanks with a sand substrate. There was no significant difference ( $P > 0.05$ ) in mean fecundity (eggs per spawn), hatch rate and larval survival rates between the two substrates. The difference in spawning rate and total egg production reflects the higher food consumption of shrimp on the hard substrate. Shrimp on a hard bottom remain active and eat day and night, while on sand they bury and will not eat during the day. Thus a lower intake of dietary nutrients appears to account for the lower rate of ovarian maturation on the sand substrate. There is no evidence from this study that a sand substrate is necessary for the ovarian maturation of eyestalk ablated *P. japonicus*.

## Introduction

The ovarian maturation of captive penaeid shrimp is influenced by a number of environmental conditions. Light quality, light intensity, photoperiod and temperature effects are most commonly investigated and have been studied for a number of species. For example, concurrently increasing water temperature and daylength promotes vitellogenesis in the Kuruma shrimp *Penaeus japonicus* (Caubere et al. 1979; Laubier-Bonichon and Laubier 1979).

The effect on shrimp ovarian maturation of varying the substrate in maturation tanks has been investigated for only a few species. The eggs of eyestalk ablated *P. monodon* spawners had increased hatch rates on white than black sand (Pudadera et al.

1980), and *P. indicus* spawners increased in fecundity and had higher spawning rates in black than white tanks (Emmerson 1980). Primavera (1985), in her exhaustive review of penaeid maturation, claimed that a soft substrate is required for the maturation of burrowing species such as *P. japonicus* but cited no reference to support this.

The present study was undertaken to determine the effect on reproductive success of a hard versus a soft sand substrate in *P. japonicus* maturation tanks. Spawning frequency, fecundity, egg hatching rates and survival of larvae to the first feeding stage were compared for shrimp held on hard and sand substrates.

### Materials and Methods

Experimental animals were collected from an earthen pond at a local shrimp farm (Moreton Bay Prawn Farm, Cleveland, Queensland) in May/June. Ten unilaterally eyestalk ablated females, ten unablated females and ten males were individually eye-tagged and carapace marked, weighed and randomly assigned to each of two fiberglass maturation tanks at a stocking density of approximately 5 shrimp·m<sup>-2</sup>. Males collected weighed 23.1-31.9 g (mean = 27.4 g) while females weighed 26.9-40.5 g (mean = 34.0 g).

Maturation tanks were circular measuring 2.8 m in diameter with 0.8 m water depth and approximately 4.5 x 10<sup>3</sup> l in volume. These tanks were colored with an inner layer of arctic blue gel coat (Fiberglass International Iso-gel Cat. No. 036). One maturation tank was provided with an undergravel filter with air-lift recirculation and sand substrate to a depth of 5 cm, and the other tank with no substrate or undergravel filter. To prevent thermal stratification, water was circulated in the hard bottom tank with a single, central air stone. Both groups of 30 shrimp were fed a measured diet of squid mantle (*Loligo chinensis*), New Zealand green mussel (*Perna canaliculatus*) and pipi (*Donax deltooides*) which were offered alternately and to excess at 0900 and 1700 hours daily. Any uneaten food was removed and recorded prior to the next feed.

Light was provided by suspended fluorescent fittings wrapped in green 70% shade cloth (Dindas Lew Cat. No. 5C7036BL) to reduce light intensity to 5 lux as measured at the water surface using a

Licor light meter (Model LI-185B) fitted with a photometric sensor (Licor Model PH4432). Daylength was controlled to 14.5L:9.5D. Water temperature was held at 28°C and water exchanged in both tanks by flow through at 150% per day.

Ovarian development was monitored daily using a submerged flashlight to reveal the shadow of the ovary on the dorsal exoskeleton. Mature females were removed and spawned on 0.25-m<sup>3</sup> rectangular drums in 150 l of water at 28°C. Egg and larvae numbers were estimated by vigorously stirring the spawning drums and counting four 100-ml subsamples. Larvae were left to develop in the spawning drum and counted at the first nauplius (N<sub>1</sub>) and first zoeal (Z<sub>1</sub>) stage.

Maturation tanks were maintained for 20 days and then surviving shrimp removed and weighed. The undergravel filter and sand were moved to the tank previously used for the hard substrate treatment and both tanks cleaned and restocked with fresh animals. The experiment was repeated for a further 20 days. In this second experiment males used weighed 23.1-32.9 g (mean = 29.4 g), and females weighed 28.3-44.6 g (mean = 36.2 g).

Fecundity, hatch rate and rate of larval survival from first nauplius to first zoea were subjected to analysis of variance to isolate time, tank and treatment effects. The covariates, spawner weight and spawn number (first spawn, second spawn, etc., for successive spawns), were included in these analyses where shown to be appropriate by linear regression analysis. Food consumption per day and change in shrimp weight over the 20 days in both tanks and for both times were also subjected to analysis of variance.

## Results

### *Spawning*

None of the unablated females spawned on either of the two substrates. The frequency of spawning of ablated females on both substrates is summarized in Table 1. Ablated females spawned 99 times, with 57 spawns being assayed. Thirty-seven out of 40 females survived ablation and the hard bottom tanks produced 65 spawns compared to 34 spawns from the sand bottom tanks. All of the ablated females on the hard substrate spawned at least once, up to a

Table 1. Summary of the spawning rates of eyestalk ablated *P. japonicus* on sand and hard substrates.

	Substrate	No. of females ablated	No. surviving ablation	No. of females that spawned	Total spawns produced	No. of spawns assayed	No. of spawns per shrimp*
Time <sub>1</sub>	Sand	10	9	6	16	6	1.78
	Hard	10	10	10	34	16	3.40
Time <sub>2</sub>	Sand	10	9	7	18	10	2.00
	Hard	10	9	9	31	25	3.44

\* Total spawns produced divided by number of shrimp surviving ablation

maximum of five spawns for one individual. On the sand substrate, 13 out of 18 ablated females spawned at least once, up to a maximum of three spawns for five individuals.

Mean fecundity, hatch rates and larval survival rates from the two substrates are shown in Table 2. From the assayed spawns the hard bottom tanks produced  $6.26 \times 10^6$  eggs,  $3.56 \times 10^6$   $N_1$  and  $2.56 \times 10^6$   $Z_1$ , while the sand bottom tanks produced  $1.72 \times 10^6$  eggs,  $1.95 \times 10^6$   $N_1$  and  $1.23 \times 10^6$   $Z_1$ .

For both experiments and both substrates there was a consistent negative relationship between spawn number and fecundity (Fig. 1a). There was no significant difference ( $P > 0.05$ ) in this relationship

Table 2. Mean fecundity, hatch rate and larval survival from assayed spawns of *P. japonicus* held on sand and hard substrates.

	Substrate	Mean number of eggs*	Mean HR# (%)	Mean HR (%) <sup>+</sup>	Mean survival to $Z_1$ (%)
Time <sub>1</sub>	Sand	198 600 (153 700 - 255 400)	70.7	81.2	77.9
	Hard	140 600 (79 100 - 233 500)	56.0	84.9	73.2
Time <sub>2</sub>	Sand	153 300 (72 500 - 270 900)	71.9	76.5	52.2
	Hard	160 300 (62 000 - 293 000)	57.4	70.9	71.4

\* range in parentheses

# hatch rate

+ does not include unfertilized spawns

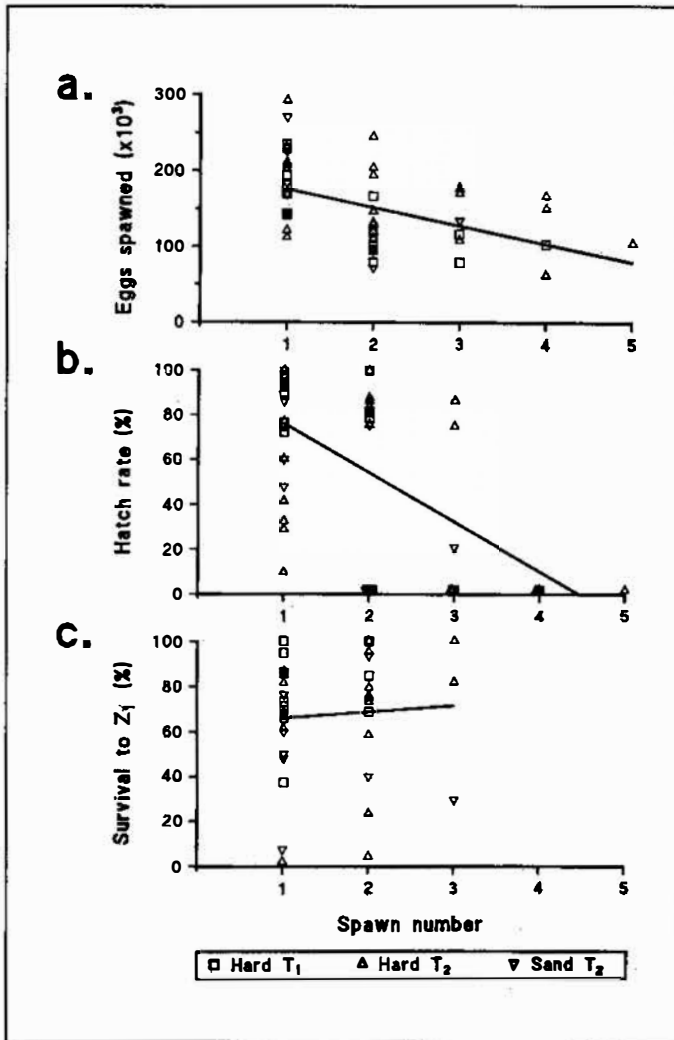


Fig. 1. Successive spawnings of *P. japonicus* - the relationship between spawn number and a. Fecundity, b. Hatch rate, c. Survival of larvae to  $Z_1$ .

either between substrates or between experiments. Data from the four tanks were therefore pooled and the relationship between spawn number and fecundity was statistically significant ( $P < 0.01$ ).

Similarly there was a negative relationship between spawn number and egg hatching rate with data from both substrates and both experiments poolable ( $P < 0.05$ ). The relationship between spawn number and hatch rate was statistically significant ( $P < 0.05$ ) (Fig. 1b).

The rate of survival of larvae to first zoea was unrelated to spawn number ( $P>0.05$ ) (Fig. 1c). The weight of each spawner was related both to its fecundity ( $P<0.01$ ) (Fig. 2a) and to egg hatch rate ( $P<0.05$ ) (Fig. 2b).

As larval survival was not related to either spawn number or spawner weight, survival to  $Z_1$  on hard and sand substrates was compared by analysis of variance. There was no significant difference ( $P>0.05$ ) in larval survival on the two substrates (Table 2). Spawner weight and spawn number were related to fecundity and hatch rate and so were included as covariates in an analysis of covariance with time, substrate and tank as factors. There was no significant difference ( $P>0.05$ ) between either mean fecundity or hatch rate on the hard and sand substrates (Table 2).

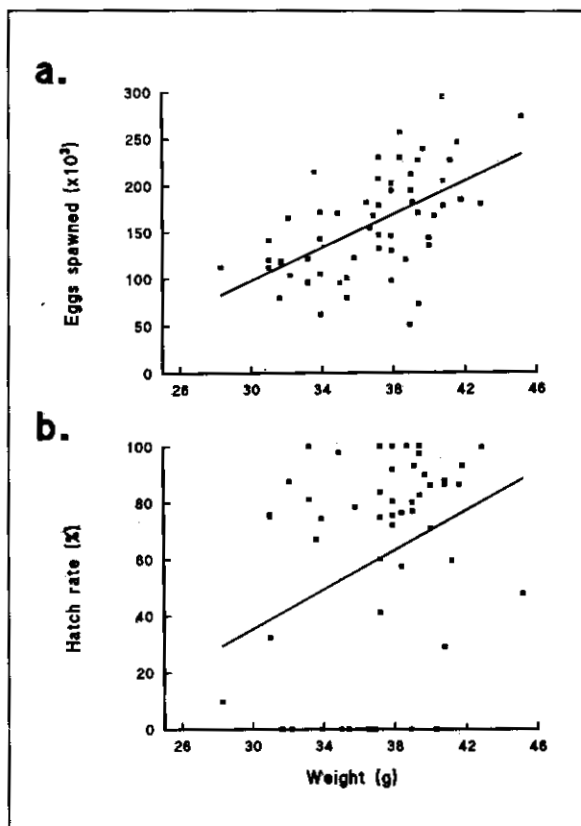


Fig. 2. The relationship between *P. japonicus* spawner weight and a. fecundity, b. hatch rate.

### **Food Consumption**

The average daily food consumption of shrimp on the two substrates is shown in Table 3.

Shrimp held on sand remained buried and did not eat during the day. Shrimp held on a hard bottom remained active and ate both night and day. Consequently, animals in the hard bottom tanks consumed an average of 123 g/day (wet weight) compared to 66 g/day for the sand bottom tanks.

Table 3. Average daily food consumption of *P. japonicus* held on sand and hard substrates.

Substrate	Time 1	Time 2	Mean (T <sub>1</sub> , T <sub>2</sub> )	LSD* (1%)
Sand	70 g	62 g	66 g	25.6 g
Hard	118 g	128 g	123 g	

\* Least significant difference

### **Weight Change**

The average weight change of the three groups of shrimp (male, ablated female and unablated female) is shown in Table 4. Male shrimp from the hard tanks gained on average 1.9 times as much weight as those on sand, while unablated females gained 2.4 times as much weight. Ablated females from the sand tanks on average maintained their weight, while those from the hard tanks lost an average 1.63% of body weight.

Table 4. Mean percentage weight change per shrimp of *P. japonicus* held on sand and hard substrates.

Substrate	Males %	Ablated females %	Unablated females %	Ablated females %*
Sand	+1.95	+0.01	+2.76	-1.16
Hard	+3.61	-1.63	+6.55	-1.63

\* Does not include females which failed to spawn



There was a significant interaction between substrate type and shrimp group on weight change ( $P < 0.05$ ) and a significant difference in the mean weight change of the three groups of animals ( $P < 0.01$ ). There was no significant difference in the starting weight within the three groups of shrimp on either substrate or between both substrates and experiments ( $P > 0.05$ ).

### Discussion

The differences in spawning rates and total egg production on a sand and hard substrate are contrary both to intuitive expectation and what has been previously reported. It appears reasonable to assume that a burrowing species such as *P. japonicus*, if held on a hard substrate, would suffer an increased level of stress. Stress has been implicated as a preventive to spawning in penaeid shrimp (Aquacop 1979; Lumare 1979; Yano, pers. comm.) and it was expected that *P. japonicus* would spawn at a higher rate in maturation tanks provided with a sand substrate. However, throughout this experiment the opposite occurred, with more females in total spawning at an increased rate in the hard bottom tanks.

Five eyestalk ablated females failed to spawn in the sand bottom tanks and it is conceivable that this reflects individual variability rather than a response to the tank substrate. If these five shrimp had spawned at the average rate of the other 13 ablated females on the sand bottom, then the hard bottom tanks would still have produced 1.3 times as many spawns in total. Thus, it appears that sand in maturation tanks may actually decrease the spawning rate of ablated *P. japonicus*, although greater replication would be needed to prove this. At the very least these results do not support the statement of Primavera (1985) that a soft substrate is required for the ovarian maturation of *P. japonicus*.

The decision whether or not to use sand in *P. japonicus* maturation tanks would likely depend on the requirements and restrictions of individual hatcheries. For example, water usage and, consequently, heating costs, are lower in a recirculating system as the rate of water exchange must be kept higher to maintain water quality in a flow-through system (Muthu and Laxminarayana 1982). One possible compromise may be to use sand but increase the hours of darkness in order to lure shrimp out to eat. However, any

decrease in daylength may tend to inhibit maturation while sudden changes in photoperiod may stress the shrimp and prevent spawning (Lumare 1979).

Another reason for using sand in *P. japonicus* maturation tanks is the reported greater mating success in sand tanks over a longer period (Hansford and McGuren 1991). However, mating in such a system would probably not exceed a 30% success rate (Lumare 1981; Hansford and McGuren 1991; present authors, unpubl. data).

It is considered that the most efficient maturation system for hatcheries unable to rely on consistent supplies of wild caught *P. japonicus* spawners would be the combined use of a large tank or small pond holding facility with a sand bottom for mating and a hard bottom maturation tank. Successfully impregnated female shrimp could be removed in early post-moult, ablated and transferred to the maturation tank. Such animals could then be expected to spawn an average two or three times before they next moulted (personal observation). The results of this study indicate that although fecundity would decrease with successive spawns (Fig. 1a), the quality of the larvae would not decrease significantly over successive fertilized spawnings (Fig. 1c). Using such a system it would be possible to maximize egg and larval production per shrimp, limiting spawner waste and thus keeping hatchery costs down.

There is an apparent correlation between the superior performance of the hard bottom tanks and the greater food consumption on that substrate. The shrimp in the hard bottom tanks ate 1.9 times as much food and produced 1.8 times as many spawns. If the mean fecundity of both tanks is adjusted for the covariates spawner weight and spawn number, the estimate of total egg production is  $10.5 \times 10^6$  eggs on the hard substrate, and  $4.88 \times 10^6$  eggs on the sand substrate. Thus the hard bottom tanks are estimated to have produced 2.15 times as many eggs as the sand bottom tanks.

The difference in food consumption is also reflected in the weight change of 'non-spawning' shrimp on the two substrates. The difference in weight gain of males and unablated females is due to sex-dependent growth rates in this species. Females in the pond at time of collection were 32% heavier on average than males in the same pond (unpubl. data). Considered in total however, the biomass increase of males and unablated females on the hard substrate is 2.1 times greater than those held on sand. The correlations between

daily food consumption, weight gain of males and unablated females and the spawning success of ablated females is summarized in Table 5.

Table 5. Ratio of food consumption, spawning success and weight gain of *P. japonicus* held on sand and hard substrates.

	Daily food consumption	Number of spawns	Total eggs spawned	Weight gain*
Ratio (sand:hard)	1:1.9	1:1.8	1:2.15	1:2.1

\* Does not include eyestalk ablated females

Ablated females generally recorded a net loss in body weight. If the females from the sand treatment that failed to spawn are disregarded, then the average weight loss of ablated females on sand was 1.16% of body weight (Table 4). This is a smaller loss than the mean loss for the hard tanks and although not statistically significant, again appears to reflect the lower spawning rates of the sand bottom tanks. Thus the ablated shrimp that spawned from the hard bottom tanks lost more weight, despite the fact that significantly more food was consumed by shrimp held on a hard bottom.

One hypothesis consistent with these observations is the existence of a threshold rate of nutrient assimilation below which secondary vitellogenesis can only continue at the expense of stored nutrient reserves. Such a nutrient deficit may in part explain the reported variable response of penaeid shrimp to attempts at rematuration (Primavera 1982; Poernomo and Hamami 1983; Yano 1984). Studies of the mobilization of nutrients in dietary stressed eyestalk ablated penaeid shrimp may enable the identification of these nutrients. Recognition and supplementation of such limiting dietary factors is essential for the development of effective maturation diets for captive penaeid shrimp.

### Acknowledgements

Thank you to the management and staff of Moreton Bay Prawn Farm for their help, and to David Mayer, Biometry Branch, QDPI for help with statistical analyses.

Funding for this project was provided by the Fishing Industry Research Development Council, Project No. 89/52.

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