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Simulated Transport of *Scylla serrata* Zoeae at Various Loading Densities

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Abstract

Percent mortality of mud crab *Scylla serrata* zoeae was determined after 6 h of simulated transport at mobile and stationary conditions at loading densities of 10, 20, 30 and 40 x 10³ ind·l⁻¹. Mortality was not significantly different among treatments immediately after transport. Surviving zoeae were stocked in basins, fed with *Brachionus rotundiformis* and mortality was compared 15 h after transport. There was no significant interaction between loading density and condition (mobile and stationary) of transport ($P > 0.05$). However, larval mortality varied significantly among densities ($P < 0.001$) regardless of the condition. A density of 10 x 10³ ind·l⁻¹ had the lowest mortality ($0.56 \pm 0.76\%$) followed by 20 x 10³ ($1.28 \pm 0.39\%$), 30 x 10³ ($4.3 \pm 0.25\%$), and 40 x 10³ ($4.3 \pm 0.31\%$) ind·l⁻¹. In another experiment, the effect of transport duration was determined at a constant loading density of 10 x 10³ ind·l⁻¹ in control (not subjected to packing and transport), shaken and unshaken conditions. Zoea mortality did not differ significantly ($P > 0.05$) after the 6, 9, and 12 h transport. Regardless of the duration, mortality was lowest in the control ($0.41 \pm 0.05\%$) compared to those in the shaken ($0.99 \pm 0.13\%$) and unshaken ($0.79 \pm 0.12\%$) conditions. Likewise, the condition but not the duration of transport affected larval survival at 15 h post-transport. Mortality was lower in the shaken ($1.92 \pm 0.22\%$) than in the unshaken condition ($2.46 \pm 0.17\%$). Since mortality is low even at 20 x 10³ ind·l⁻¹, this can still be used to transport *S. serrata* zoeae for 6 h. However, loading density should be reduced to 10 x 10³ ind·l⁻¹ for transport duration up to 12 h.

Introduction

Pond-reared *Scylla serrata* (555 to 665 g body weight) from Samar and Capiz, Philippines that were maintained at SEAFDEC/AQD produced hatchlings ranging from 1.2 to 3.5 x 10⁶ zoeae. When eggs of more than one female hatch within the same period and a hatchery can no longer accommodate all the zoeae, transporting them to other hatcheries can be considered. In *Penaeus monodon*, transport of wild gravid females is sometimes stressful resulting to spawning failure. When prawn hatcheries have an excess supply of larvae from ablated spawners, transport of nauplii to other hatcheries is commonly practiced (Primavera 1989). In Taiwan, a number of hatchery operators obtain nauplii from brokers (Liao 1986). Hence, for both

mud crab and prawn obtaining larvae from elsewhere and transporting them to a hatchery partly assures the operators that they can have an ample supply of larvae to rear.

Improper handling and transport of animals could lead to high mortalities (Gillespie and Burke 1996). Thus, optimal conditions of transport must be determined to avoid unnecessary mortalities. This paper reports the optimal loading density and duration of transport for *S. serrata* zoeae.

Methodology

Loading density

Newly hatched (2 to 4 h post-hatch) unfed zoeae from a female held in a maturation tank at SEAFDEC/AQD were obtained. To serve as a reference standard, 5×10^3 zoeae (0.91 mm total length or TL) were counted individually and stocked in a basin with 500 ml seawater (32 ppt). About 5×10^3 zoeae were stocked in other basins through visual comparison with the reference standard. Zoeae from these basins were appropriately combined to obtain densities of 10, 20, 30, and 40×10^3 ind·l⁻¹. The basins were partially covered to allow the zoeae to concentrate on the lighted area. Excess water was siphoned out in areas where lesser larvae concentrated until a final volume of one liter was obtained. Zoeae were packed in 34 x 25 cm plastic bags that were placed in basins with cool seawater (24 to 25 °C). The bags were then inflated with oxygen and sealed using rubber bands when water temperature in both bags and basins were similar. Since actual transport of zoeae consists of mobile and stationary periods, shaken and unshaken conditions were included as a factor in addition to the different loading densities. For the shaken condition, bags were loaded in a styrofoam box and placed on an orbit shaker at 75 oscillations per minute over a 6-h period to simulate transport. A separate set of the same batch remained unshaken. Air temperature throughout the experiment ranged from 24 to 26°C. Dissolved oxygen, nitrite and ammonia levels were measured before inflating the bags with oxygen and immediately after simulated transport.

After 6 h, zoeae in each bag were placed in basins and dead ones were counted. Survivors were stocked in plastic containers with 6 l of seawater at about 10×10^3 ind per container with moderate aeration. Since the stocking density was high, 0.5 ppm furazolidone was applied to prevent proliferation of luminescent bacteria. Zoeae were fed rotifer *Brachionus rotundiformis* at 10 to 15 ind·ml⁻¹. After another 15 h, mortality rates were determined. Two separate trials were conducted with three replicates for each loading density.

To further determine the effect of food deprivation, zoeae subjected to simulated transport from similar loading densities were combined and stocked in 1.5 ton tanks at 50 ind·l⁻¹ and their performance was compared with zoeae of the same batch that were fed immediately after hatching and not subjected to transport. Larval rearing protocol described by Quintio et

al. (this volume) was followed. Two trials were done with three replicates for each treatment.

Duration

Using the best stocking density of 10×10^3 ind·l⁻¹, another batch of newly hatched unfed zoeae were used to determine survival after 6, 9 and 12 h at mobile and stationary conditions. Packing procedure and rearing conditions were the same as in the loading density experiment. Another set of zoeae of the same batch stocked directly in aerated containers at 10×10^3 ind·l⁻¹ for 6, 9 and 12 h served as the control. After each transport duration, surviving zoeae were stocked in containers with *B. rotundiformis* at 10 to 15 ind·ml⁻¹ and mortality rates were determined after another 15 h excluding the control. There were three replicates for each treatment.

Statistical analysis

Percentage mortality was transformed to arcsin values prior to analysis of variance. Treatment means were compared using Duncan's new multiple range test (Walpole 1982; Gomez and Gomez 1984).

Results

Loading density

Immediately after 6 h of simulated transport, less than 10 dead larvae were counted in all containers. At 15 h post transport, a two-way analysis of variance of zoeae mortality revealed that loading density but not condition of transport affected mortality. No interaction between the two factors was detected ($P > 0.05$) hence, means were pooled and compared. Larval mortality at 15 h post-transport varied significantly among loading densities ($P < 0.001$) regardless of transport condition. A loading density of 10×10^3 had the lowest mortality ($0.56 \pm 0.07\%$) followed by 20×10^3 ($1.28 \pm 0.39\%$), 30×10^3 ($4.3 \pm 0.25\%$), and 40×10^3 ind·l⁻¹ ($4.3 \pm 0.31\%$) (Fig.1). When larvae from the transport experiment were combined and stocked in 1.5 ton tanks, the survival rate ($4.37 \pm 1.07\%$; zoea 1 to megalopa) was comparable to the same batch of larvae ($4.75 \pm 1.2\%$) that were not subjected to transport. The duration from zoea 1 to megalopa was 17 to 19 days in both treatments.

Dissolved oxygen levels ranged from 5.2 to 5.4 ppm prior to inflating the bag and from 24 to 26 ppm immediately after transport. Initial nitrite and ammonia ranges were 0.07 to 0.08 ppm and 0.10 to 0.14 ppm, respectively. After transport, higher nitrite and ammonia were noted (Table 1). Lowest ammonia and nitrite levels were recorded at 10×10^3 ind·l⁻¹ in shaken condition. No significant differences were noted in all other treatments.

Duration

Zoeae mortality did not differ after the 6, 9 and 12 h transport duration. However, the condition of transport affected the survival of zoeae. Mortality was lowest in zoeae that were not subjected to packing and transport (control, $0.41 \pm 0.05\%$) compared to those in the shaken ($0.99 \pm 0.13\%$) and unshaken ($0.79 \pm 0.12\%$) conditions (Fig. 2A). Likewise, condition not the duration of transport affected larval survival at 15 h post-transport. Mortality was lower in shaken ($1.92 \pm 0.22\%$) than in the unshaken condition ($2.46 \pm 0.17\%$) (Fig. 2B).

Discussion

The increase in ammonia and nitrite levels after transport reflects nitrogen excretion as nutrient reserves were utilized. Information on the optimum ammonia and nitrite levels for crab larvae are still lacking. In a study conducted at SEAFDEC/AQD (unpublished manuscript), *S. serrata* zoea could tolerate 3 ppm ammonia for three days. Ammonia levels up to 6 ppm at pH 7.5 to 8.0 can be tolerated by *P. monodon* postlarvae (Noor-Hamid et al. 1994). In contrast, postlarvae of *Homarus americanus* are

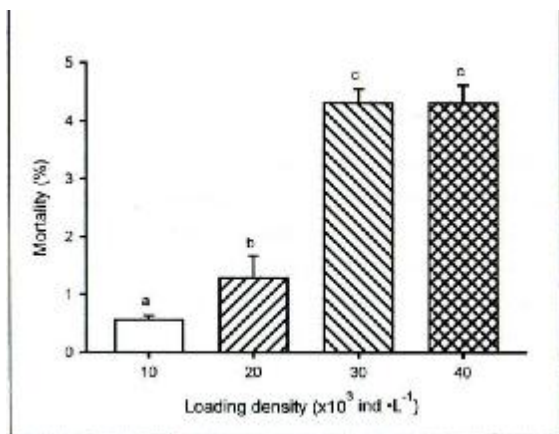


Fig. 1. Mortality (mean \pm SE) of *S. serrata* zoeae 15 h after 6 h of simulated transport at different loading densities

Table 1. Ranges of selected physico-chemical parameters of transport water containing zoeae subjected to 6 h of shaken and unshaken conditions

Loading density (x 10 ³ ind.l ⁻¹)	pH	NO ₂ -N (ppm)	NH ₃ -N (ppm)
Unshaken			
10	7.0	0.74 – 1.24	0.31 – 0.95
20	7.0 – 7.7	0.46 – 1.04	0.48 – 0.67
30	7.7 – 7.8	0.55 – 0.88	0.72 – 0.89
40	7.6 – 7.7	0.35 – 0.89	0.62 – 1.01
Shaken			
10	7.8 – 7.9	0.10 – 0.13	0.12 – 0.16
20	7.9	0.56 – 0.79	0.39 – 0.59
30	7.8	0.82 – 1.39	0.96 – 1.39
40	7.6 – 7.8	0.35 – 0.94	0.62 – 1.42

quite sensitive to unionized ammonia ($\text{NH}_3\text{-N}$) that has an incipient LC_{50} of 1.4 ppm and is relatively toxic at high temperature and pH (Ennis 1995). Since mortality is very low (4.3%) even at high loading densities, exposure of zoeae to 1.42 ppm $\text{NH}_3\text{-N}$ and 1.39 ppm $\text{NO}_2\text{-N}$ for a few hours is possibly not very critical for their survival. Crowding associated with handling and packing in both unshaken and shaken conditions was likely the cause of mortality. Additional stress to zoeae was the impact of shaking during simulated transport. This was further confirmed in the second experiment where zoeae survived better when they were not subjected to packing and shaking.

Brachyuran and anomuran larvae must feed after hatching in order to start the molt cycle (Anger and Dawirs 1981; Wehrtmann 1991) while penaeid larvae subsist on yolk stored in their bodies after hatching. The survival, metamorphosis and ingestion rates of newly hatched *S. serrata* zoeae starved for 24 h were comparable to those fed immediately after hatching (Lumasag and Quintio 1998). It is possible that there are still enough nutrient reserves in zoea that can be utilized when feeds are not readily available. Therefore, the 12 h transport period is feasible for zoeae after 4 h post-hatch. Proteins and lipids are consumed during starvation in the zoea of the spider crab *Hyas araneus* (Anger 1986). The greater part of yolk reserves (lipid) is used up during the first day of starvation in hermit crab zoea (Pandian and Schumann 1967). If starvation continues, protein reserves seem to be the main energy resources (Dawirs 1983).

Spawner collectors and hatchery operators of *P. monodon* transport nauplii at 200 to 400 $\times 10^3$ in 20 l plastic containers (10 to 25 $\times 10^3$ $\text{ind}\cdot\text{l}^{-1}$) without oxygenation or in oxygenated plastic bags at ambient temperature for 4 to 5 h overland (Primavera 1989). Loading density is reduced to 100 $\times 10^3$ nauplii/container for transport over 5 h. The optimal loading density (10 $\times 10^3$ $\text{ind}\cdot\text{l}^{-1}$) in the present study is within the range of what has been practiced for *P. monodon*. Considering that *P. monodon* nauplii (N_I to N_{IV} : 0.30 to 0.58 mm TL) are smaller than *S.*

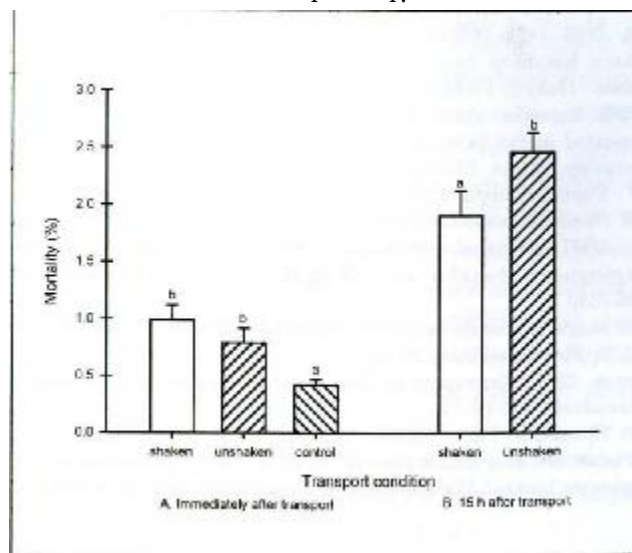


Fig. 2. Mortality (mean \pm SE) of *S. serrata* zoeae (A) immediately and (B) 15 h after 6, 9, and 12 h of simulated transport at various conditions at 10×10^3 $\text{ind}\cdot\text{l}^{-1}$

serrata zoeae 1 (0.91 mm TL, 0.65 mm body length or BL), the former can be loaded up to 25×10^3 ind·l⁻¹.

In an earlier report, *S. serrata* megalops (3.54 mm BL) can be optimally loaded at 50 ind·l⁻¹ at 22 to 24°C up to 9 h of transport (Quinitio and Parado-Esteva 2000). Due to their small size and less cannibalistic nature compared to megalops, zoeae can be loaded up to 10×10^3 ind·l⁻¹ for 6 h. Zoeae are more resistant than megalops due to their smaller mass as lesser energy would be imparted to them upon impact of moving water.

A loading density of up to 20×10^3 ind·l⁻¹ is recommended to transport *S. serrata* zoeae for 6 h at 24 to 25°C. For transport duration up to 12 h, loading density should be reduced to 10×10^3 ind·l⁻¹.

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