Effect of Stocking Density on Survival Rate and Bacterial Compositions in the Larval Rearing of the Giant Freshwater Prawn *(Macrobrachium rosenbergii)*

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Abstract

Larval rearing of the giant freshwater prawn, *Macrobrachium rosenbergii* using a clear-water system was studied at a commercial prawn hatchery located in Ratchaburi province, Thailand. The prawn larvae were stocked in circular fiberglass tanks containing 2.5 cubic meters (m^3) of 15 parts per thousand (ppt) seawater at the density of 100,000/ m^3, 120,000/ m^3 and 140,000/ m^3. Water quality parameters and quantitative and qualitative analyses of bacterial populations in the water and postlarvae were studied. The average survival rates of postlarvae in the 100,000/ m^3, 120,000/ m^3 and 140,000/ m^3 groups were 91%, 87% and 74%, respectively. There were significant differences among the three density groups. The water quality throughout the rearing period was suitable for nursing postlarvae. The average total numbers of bacteria in water at the stocking densities of 100,000/m^3 and 120,000/m^3 ranged from $3.20x10^3$ to $4.40x10^4$ and $4.27x10^3$ to $7.6x10^3$ CFU/ml, respectively, and were significantly lower than for the 140,000/ m^3 group in which the average total bacteria ranged from $9.04x10^3$ to $9.00x10^4$ CFU/ml. The average total numbers of bacteria in postlarvae at densities of 100,000/m^3, 120,000/$m^3$and 140,000/m^3 were $8.94x10\pm9.77x10$, $1.36x10^2\pm1.39x10^2$ and $2.04x10^2\pm2.04x10^2$ CFU/g, respectively. Five species of bacterial flora were identified from the water and postlarvae and the one most frequently isolated was the *Vibrio* community, represented by *Vibrio vulnificus, V. alginolyticus, V. cholerae* (non 01), *V. mimicus* and *Aeromonas hydrophila.*

There was an absence of mass mortalities during the 27-day rearing period in this study. No histopathological changes related to diseases were observed in any postlarvae. External protozoans such as *Epistylis* sp. were found from the body cuticular surfaces of some of the postlarvae sampled, especially in the 140,000/m^3 group. The results from this study indicated that direct involvement of bacteria alone was unlikely to cause mass mortalities of the prawn larvae. Environmental stressors from high stocking density affected the survival rate of prawn postlarvae.

Keywords : Larval rearing, Bacterial Compositions, Giant Freshwater Prawn *(Macrobrachium rosenbergii)*
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Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii,* has become one of the most widely cultured aquatic animals in Thailand. Prawn production was 2,700 tons in 1983 and had increased to more than 30,000 tons in 2004. Since Ling (1962) reported the discovery that salinity was an important basic requirement for prawn larval survival and development throughout

the early life stages, various techniques for raising prawn larvae have been developed (Menasveta and Piyatiratitivokul, 1980).

Inland hatcheries in Thailand operate by trucking seawater and brine or rock salt to the site and dilute this with freshwater (Tunsutapanich, 1981). Most hatcheries are able to produce some prawn larvae from eggs in commercial quantities. However, the major factor that limits the production of this species is the inadequate availability of seed due to diseases affecting the stock. Disease problems associated with *M. rosenbergii* include protozoan infestation (especially *Zoothamnium, Vorticella* and *Epistylis* spp.), fungal pathogens (such as *Lagenidium callinectes, Sirolpidium* spp. and *Fusarium solani*), bacterial problems following injury to the exoskeleton, and molt arrest due to poor environmental conditions or nutritional deficiencies (Johnson, 1982; New, 1990)

The most common bacterial disease of *M. rosenbergii* affects their cuticles/shells, leading to "black-spot" bacterial necrosis and gill obstruction. The bacteria isolated from infected prawns were identified as species in the genera *Vibrio, Pseudomonas, Aeromonas, Beneckea* and *Leucothrix* (Sung *et al.*, 1997; Lombardi and Labao, 1991 a,b) . Cheng and Chen (1998) reported that Enterococcus-like bacteria were associated with mortalities of prawn larvae in Taiwan, and that environmental parameters such as temperature and pH might have been important factors in the disease outbreaks. Cheng and Combes (1990) reported that physiological stress could affect defense mechanisms. For example, a significant negative correlation between phenoloxidase activity and tidal height in *Carcinus maenas,* and an increased prevalence of shell disease in marine decapod crustaceans has been reported to result from polluted environments, also suggesting a decrease in imunocompetence and survival rate (Gopalan and Young, 1975; Young and Pearce, 1975; Hauton *et al.,* 1995). Quantitative and qualitative aspects of bacterial flora associated with *M. rosenbergii* have been reported (Miyamoto *et al.,* 1983; Fujioka and Greco, 1984). However, earlier studies have been carried out on water and larval samples from different places with different rearing methods.

In the present study, we evaluated the quantitative and qualitative changes of bacterial populations in water during the larval rearing of *M. rosenbergii* with three different stocking densities, and the relationship between water quality and survival rate.

Materials and Methods

Larval rearing

This study was conducted at a prawn hatchery located in Ratchaburi province, Thailand. At the hatchery, the prawn seeds were produced by an intensive clear-water system and water was disinfected with calcium hypochlorite at a concentration of 30 ppm before use. For the rearing trials, gray- to black-egged female prawns (60-70 g in weight) collected from culture ponds were placed in the larval hatching tanks. Upon hatching, the larvae were transferred to 3 m^3 fiberglass tanks at stocking densities of 100,000/m^3, 120,000/m^3 and 140,000/ m^3, with three replicates for each group. During the 27-day course of larval rearing, larvae were fed with newly hatched *Artemia* nauplii (three times/day) during the first 9 days. From the 10th day to metamorphosis, the prawn larvae were fed with steamed eggs (three times/day). The uneaten feed and dead larvae were siphoned out from the rearing tanks, 4 hours after every feeding. The amount of feed used daily was approximately 30% of the total body weight of the larvae. Water exchange (50%) was done every 3 days from the tenth day to metamorphosis.

Table 1. The average water quality conditions during the study.

Water quality	mean ± SD		
	100,000 PL/m^3	**120,000 PL/m^3**	**140,000 PL/m^3**
Dissolved oxygen (mg/l)	5.7±0.1	5.5±0.3	5.2±0.0
Temperature (°C)	29.6± 0.1	29.3±0.3	29.2±0.3
pH	8.2±0.0	8.2±0.0	8.2±0.0
EC (mmhos/cm)	20.1±0.1	20.0±0.1	20.6±0.5
Salinity (ppt)	12.1±0.1	11.9±0.1	12.0±0.1
Total alkalinity(mg/l)	129.2±0.3	129.2±0.8	128.9±3.3
Total hardness (mg/l)	2,898±54	3,493±113	2,907±79
TAN (mg/l)	0.64±0.14	0.64±0.15	0.86±0.08
Nitrite-nitrogen (mg/l)	0.03±0.00	0.03±0.00	0.03±0.00

The numbers of aerobic heterotrophic bacteria from water during the rearing period and prawn postlarvae are shown in Table 2. The total numbers of bacteria in water varied among the three groups during the state of metamorphosis.

There was no significant difference between the average total bacterial counts in the 100,000/m^3 and 120,000/m^3 groups. However, total bacterial counts from these groups was significant lower than in the 140,000/m^3 group (Table 2). Total bacterial counts in this study were lower than those previously reported by Miyamoto *et al.* (1983); Anderson *et al.* (1989) and Phatarpekar *et al.* (2002).

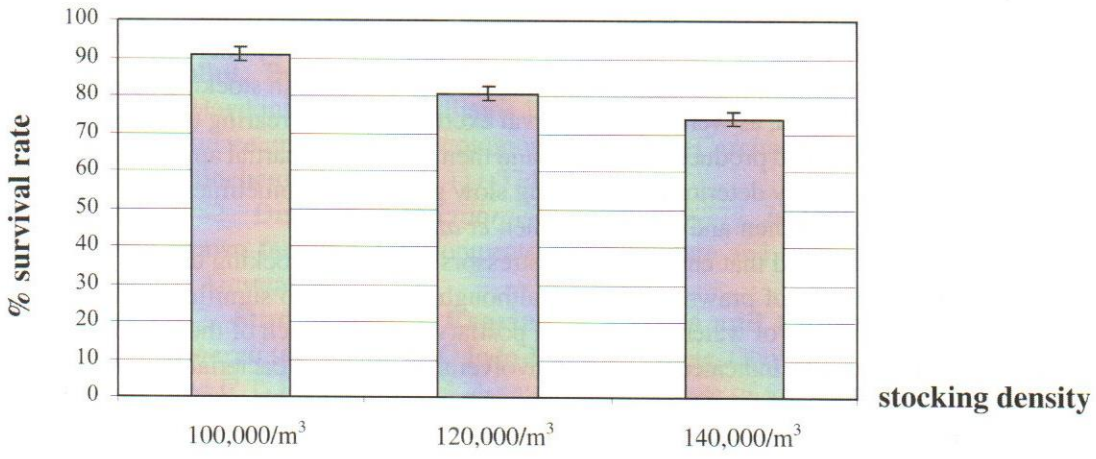
Table 2. The average total bacterial counts (mean±S.D.; n=3) of rearing water (CFU/ml) and postlarvae (PL) (CFU/g).

Days	Total bacteria count					
	100,000/m^3		120,000/m^3		140,000/m^3	
	Water	PL	water	PL	water	PL
3	$3.20x10^3±2.41x10^3$	-	$4.27x10^3±1.85x10^3$	-	$1.77x10^4±2.23x10^4$	-
6	$1.20x10^4±1.26x10^4$	-	$1.35x10^4±6.42x10^3$	-	$3.05x10^4±3.34x10^4$	-
9	$1.33x10^4±7.28x10^3$	-	$1.49x10^4±1.42x10^3$	-	$1.80x10^4±5.88x10^3$	-
12	$3.87x10^4±4.48x10^4$	-	$6.27x10^4±3.26x10^4$	-	$8.47x10^4±1.39x10^5$	-
15	$6.82x10^3±4.45x10^4$	-	$9.70x10^3±4.62x10^3$	-	$2.35x10^4±1.95x10^4$	-
18	$4.40x10^4±1.29x10^4$	-	$7.60x10^4±1.72x10^4$	-	$9.00x10^4±1.05x10^4$	-
21	$4.02x10^4±7.22x10^3$	-	$4.29x10^4±1.82x10^4$	-	$4.64x10^4±1.97x10^4$	-
24	$7.00x10^3±8.03x10^3$	-	$8.70x10^3±2.33x10^3$	-	$9.00x10^3±4.07x10^2$	-
27	$1.03x10^4±4.62x10^3$	$1.05x10^2±1.13x10^2$	$1.70x10^4±6.61x10^3$	$2.25x10^2±2.59x10^2$	$1.73x10^4±5.52x10^3$	$3.67x10^2±2.52x10^2$
average	$9.88x10^3±2.92x10^3$	-	$1.28x10^4±3.50x10^3$	-	$2.14x10^4±8.03x10^3$	-

Table 3. Relative frequency of bacteria recovered from larval rearing water and postlarvae (mean±S.D.; n=3).

Bacteria	100,000/m^3		120,000/m^3		140,000/m^3	
	water	PL	water	PL	water	PL
Aeromonas hydrophila	$4.34x10^2±2.88x10^2$	2.67x10±2.08x10	$6.05x10^2±5.34x10^2$	6.33x10±4.62x10	$1.31x10^3±1.25x10^3$	$1.05x10^2±1.13x10^2$
Vibrio vulnificus	$1.56x10^3±1.88x10^3$	$1.79x10^2±1.53x10^2$	$2.37x10^3±1.44x10^3$	$2.66x10^2±3.42x10^2$	$2.62x10^3±1.86x10^3$	$3.94x10^2±2.79x10^2$
V. alginolyticus	$4.68x10^2±3.94x10^2$	2.33x10±1.53x10	$7.76x10^2±5.03x10^2$	3.00x10±1.73x10	$1.09x10^3±7.29x10^2$	4.50x10±2.14x10
V. cholerae (non 01)	4.15x10±3.04x10	3.08x10±1.17x10	6.25x10±4.17x10	3.15x10±2.25x10	9.04x10±4.37x10	4.10x10±1.99x10
V. mimicus	1.95x10±1.34x10	0.88x10±0.61x10	7.00x10±3.82x10	1.58x10±0.48x10	9.40x10±4.10x10	2.73x10±1.66x10
Total bacteria	$9.88x10^3±2.92x10^3$	8.94x10±9.77x10	$1.28x10^4±3.50x10^3$	$1.36x10^2±1.39x10^2$	$2.14x10^4±8.03x10^3$	$2.04x10^2±2.04x10^2$

Fig. 1. The average survival rate of the postlarvae.



The average total numbers of bacteria in postlarvae at the densities of 100,000/m^3, 120,000/m^3 and 140,000/m^3 were lower than those reported by Phatarpekar *et al.*(2002); Miyamoto *et al.*(1983), Anderson *et al.*(1989) and Sahul Hameed (1993) reported a gradual increase in the total numbers of bacteria from eggs to postlarvae. Phatarpekar *et al.* (2002) noted that as long as physico-chemical parameters are maintained within the normal range and healthy rearing techniques are practiced, high bacterial populations could be a result of factors such as intensive feeding. The lower bacterial counts reported in this study might have been because the rearing techniques were different from those used in previous studies. The present investigation used a clear-water system in which the water was disinfected with calcium hypochlorite at a concentration of 30 ppm before use and the rearing water was exchanged 50% every 3 days from day 10 to metamorphosis.

Five species of bacterial flora were identified from the rearing water and prawn postlarvae (Table 3). Most frequently isolated were *Vibrio* bacteria represented by *Vibrio vulnificus, V. alginolyticus, V. cholerae* (non 01) and *V. mimicus. Aeromonas hydrophila* was also observed in the rearing water and prawn postlarvae. In this study, the bacterial species isolated from rearing water were the same as those in the postlarvae. Our results were similar to those in earlier reports by Anderson *et al.* (1989) and Sung and Hong (1997). *Vibrio* was found to be the dominant taxon in the water and postlarvae, similar to the report of Sahul Hameed (2003). Phatarpekar *et al.* (2002) reported that pathogenic bacteria could enter hatchery systems from three principal routes: rearing water, broodstock and feeds. Skjermo and Vadstein (1999) noted that those pathogenic bacteria were common in seawater and took advantage of ecological changes which occurred when the water was used in hatcheries. Colorni (1985) reported rich bacterial flora associated with the prawns' first feed, nauplii of the brine shrimp, *Artemia salina,* in the digestive tract of 1- to 30-day-old prawn larvae. However, experimental infection with massive concentrations of bacterial monocultures did not show any significant differences from the control.

Histopathological examination did not reveal any abnormalities in all the sections examined. Epibiont fouling with *Epistylis* sp. was found on the body cuticular surfaces of some postlarvae sampled, especially the 140,000/m^3 group. However, the presence of *Epistylis, Zoothamnium* and *Lagenidium* in the hatchery probably represents no more than a mild nuisance and is not responsible for causing mass larval mortalities (Cook, 1971; Lightner and Fontaine, 1973; Colorni 1985).

The highest survival rate of postlarvae in this study was from a stocking density of 100,000/m^3 (91.00±2.00%), and lowest was at the stocking density of 140,000/m^3 (74.00±2.00%) (Fig. 1). There were significant differences ($P<0.05$) among the three density groups.

The postlarval survival rate seemed to have been affected by increasing densities. The lowest survival rate was in the highest stocking density group.High stocking density results in increasing inputs of feed, as well as prawn larval excretions, in the rearing tanks. Subsequent degradation of leftover feed produces ammonia, and then nitrite from partial ammonia oxidation, which lead to water quality deterioration, causing slow growth and sometimes high mortalities (Blackburn *et al.,* 1988; Chen and Lin 1992; Chen *et al.,* 1994).

It could be concluded that environmental stressors from high stocking density negatively affected the survival rate of prawn postlarvae, although there was no significant difference in the bacterial compositions of water samples and postlarvae among each of the stocking density groups. The study's results indicated that direct involvement of normal bacterial flora alone could not cause mass mortality of the prawn larvae. In order to achieve a high survival rate for larval rearing of *M. rosenbergii,* the stocking density of the larvae should not exceed 120,000/m^3.

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