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Appropriate Postlarval Age and Stocking Densities of *Litopenaeus vannamei* Boone for Rearing in Low Salinity Water

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ABSTRACT

The objective of this study was to evaluate under laboratory conditions the survival and growth rate of postlarvae (PL, where PL_x represents the age at day *x*) at the stages of PL_8 and PL_{10} of *Litopenaeus vannamei* during 62 d of rearing in water with low salinity levels of 1 and 3 parts per thousand (ppt) with three different stocking densities. The experiment consisted of 12 treatments with three replicate tanks per treatment: treatment 1 (1 ppt, PL_8, 60 PL/m^2); treatment 2 (1 ppt, PL_8, 90 PL/m^2); treatment 3 (1 ppt, PL_8, 120 PL/m^2); treatment 4 (1 ppt, PL_{10}, 60 PL/m^2); treatment 5 (1 ppt, PL_{10}, 90 PL/m^2); treatment 6 (1 ppt, PL_{10}, 120 PL/m^2); treatment 7 (3 ppt, PL_8, 60 PL/m^2); treatment 8 (3 ppt, PL_8, 90 PL/m^2); treatment 9 (3 ppt, PL_8, 120 PL/m^2); treatment 10 (3 ppt, PL_{10}, 60 PL/m^2); treatment 11 (3 ppt, PL_{10}, 90 PL/m^2); and treatment 12 (3 ppt, PL_{10}, 120 PL/m^2). Each 500-L fiberglass tank was equipped with adequate air diffusion and a submersible heater to maintain the temperature at $29\pm1^{o}C$. Shrimp were fed a commercial feed four times daily. Water samples were analyzed for concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+ , Cl^- and SO_4^{2-} ions every 10 d. After 62 d, shrimp were harvested. There were significant (P<0.05) differences in survival rates of shrimp between postlarvae reared in water with a salinity of 1 ppt and 3 ppt. The survival and growth rates of treatments 7-12 (PL_8 and PL_{10}, 60-120 PL/m^2) reared in a salinity of 3 ppt were 80.9%, and 0.034 g/d, respectively. However, there were significant (P>0.05) differences in the survival rate and final weights of shrimp with either PL_8 or PL_{10} at the same stocking rate and reared in water with a salinity level of 1 or 3 ppt. The concentrations of six major ions (Mg^{2+}, Ca^{2+}, Na^+, K^+, Cl^- and SO_4^{2-}) in water with a salinity level of 3 ppt (treatments 7-12) were significantly (P<0.05) higher than those in water with a salinity level of 1 ppt (treatments 1-6) throughout the experiment. The results of the present study indicated that in order to achieve good survival and growth of *L. vannamei*, PL_8-PL_{10} should be stocked with a density of not more than 60 PL/m^2 at 1 ppt or at a stocking rate between 60 and 90 PL/m^2 at 3 ppt.

Keywords: *Litopenaeus vannamei*, low salinity water, ion concentrations

INTRODUCTION

Since 1991, Thailand has been the world's leading shrimp exporter, with black tiger shrimp (*Penaeus monodon*) being the most common cultured marine shrimp from the 1990s until 2004 (Limsuwan and Chanratchakool, 2004). However, since the introduction of the Pacific white shrimp (*Litopenaeus vannamei*) into the country, shrimp farming in Thailand has been undergoing a dramatic transformation. Currently, Pacific white shrimp is rapidly replacing the black tiger shrimp

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as the main farmed species. The main reason for this change is that *L. vannamei* has a faster growth, higher stocking rate and yield, and incurs lower production costs than *P. monodon*.

The culture of Pacific white shrimp has been widespread along the eastern coast of the Pacific Ocean from Mexico to northern Peru (Holthuis, 1980; Perez and Kensley, 1997) and is the most commonly cultured shrimp in the western hemisphere (Rosenberry, 2000). This species inhabits and is being cultured in coastal waters with a salinity range from 1 to 40 ppt (Bray *et al.*, 1994; Somocha *et al.*, 1998; Mc Graw *et al.*, 2002). In the United States, farmers in Alabama, Arizona, Mississippi, South Carolina and Texas raise *L. vannamei* in low salinity well water. Despite the relative success of farmers in culturing *L. vannamei* in inland low salinity waters, problems still arise from deficiencies in the ionic profiles of pond water (Atwood *et al.*, 2003; Saoud *et al.*, 2003), due to different salinities and different ionic compositions (Boyd and Thunjai, 2003). The lack of necessary essential ions, including potassium (K^+) and magnesium (Mg^{2+}), has been proven to limit the growth and survival of shrimp (Saoud *et al.*, 2003; Davis *et al.*, 2005).

In Thailand, shrimp farmers use low-salinity water from two sources, either from brackish water in streams or rivers, or brine water (100-200 ppt salinity). Shrimp farmers dilute brine water with freshwater to a salinity level of 3-5 ppt for stocking postlarvae (PL_8-PL_{10}) and then add more freshwater during the culture period, so that the salinity is decreased to 1-2 ppt by the time the shrimp are harvested (Limsuwan, 2000; Szuster and Flaherty, 2002; Limsuwan and Chanrat-chakool, 2004). Several studies have demonstrated normal growth and survival of intensively farm-reared *L. vanamei* if the salinity of the water is maintained not lower than 4 ppt throughout the culture period (Prawitwilaikul, 2004; Limhang, 2005). Many small-scale shrimp farmers in Thailand, who have used PL_8 to PL_{10} for stocking in low salinity waters, have faced problems related to poor survival and growth, particularly with inland water with a salinity lower than 4 ppt. Most farmers have solved this problem by increasing the stocking level of PL to compensate for the poor survival and growth rate but results have been unpredictable. In order to solve this problem, the appropriate stocking rate for different PL ages and salinity levels should be determined. The short-term (48 h) effect of various low salinity levels on the survival rate of different postlarval ages of *L. vannamei* ($PL_{10, 15, 25}$) was studied by Davis *et al.* (2002). Saoud *et al.* (2003) suggested that although short term bioassays are a good way to quickly screen waters, longer term studies should be conducted as well. However, the long-term effects of various ionic profiles of low salinity water of 1-3 ppt on the survival and growth rates of PL_8 to PL_{10} *L. vannamei* have not been studied. The objective of the present study was to evaluate the survival and growth rate of PL_8 and PL_{10} *L. vannamei* during a 62-day rearing period in water with low salinity levels of 1 and 3 ppt with three different stocking densities.

MATERIALS AND METHODS

The study was conducted at the Aquaculture Business Research Center (ABRC), Faculty of Fisheries, Kasetsart University.

Low salinity water

Low salinity water samples with salinity levels of 1 and 3 ppt were prepared two weeks prior to the experiment, using brine water with a salinity level of 100 ppt that was diluted with dechlorinated tap water in fiberglass tanks (5,000 L) to salinity levels of 1 and 3 ppt.

Experimental specimens

Postlarval *L. vannamei*, PL_5 were obtained from a hatchery in Chachoengsao province, Thailand. After transportation of the PL to ABRC, the animals were acclimated from 15 ppt to low salinity levels of 1 and 3 ppt over a period between three and five days in a 500-L fiberglass tank for each salinity level. During the acclimation period, the PL were fed with a combination of *Artemia nauplii* and a commercial feed until they reached PL_8 and PL_{10} for the commencement of the growth and survival trial.

Experimental design

The experiment consisted of 12 treatments with three replicate tanks per treatment, based on postlarval age (two levels) and stocking density (three levels) in water of two levels of salinity. A 2 × 2 × 3 factorial design was used. The treatments were:

Treatment 1. PL_8 were stocked at a rate of 60 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 2. PL_8 were stocked at a rate of 90 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 3. PL_8 were stocked at a rate of 120 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 4. PL_{10} were stocked at a rate of 60 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 5. PL_{10} were stocked at a rate of 90 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 6. PL_{10} were stocked at a rate of 120 PL/m^2 in tanks with a salinity level of 1 ppt.
Treatment 7. PL_8 were stocked at a rate of 60 PL/m^2 in tanks with a salinity level of 3 ppt.
Treatment 8. PL_8 were stocked at a rate of 90 PL/m^2 in tanks with a salinity level of 3 ppt.
Treatment 9. PL_8 were stocked at a rate of 120 PL/m^2 in tanks with a salinity level of 3 ppt.
Treatment 10. PL_{10} were stocked at a rate of 60 PL/m^2 in tanks with a salinity level of 3 ppt.
Treatment 11. PL_{10} were stocked at a rate of 90 PL/m^2 in tanks with a salinity level of 3 ppt.
Treatment 12. PL_{10} were stocked at a rate of 120 PL/m^2 in tanks with a salinity level of 3 ppt.

The experiment was conducted in 36 fiberglass tanks, each holding 500 L. Each tank was equipped with an adequate air diffuser and a submersible heater to maintain a temperature of 29 ± 1°C. During the first week, the PL were maintained on a combination of *Artemia nauplii* and commercial feed. Thereafter, shrimp were fed a commercial feed four times daily at 08:00, 13:00, 18:00 and 22:00. Shrimp in each tank were appraised weekly and their feed ration was adjusted accordingly. All experimental tanks were covered with green nets to regulate the light intensity to optimal conditions for shrimp feeding. The tank water was changed weekly at the same rate for all treatment groups. Levels of dissolved oxygen (DO), pH, salinity and the temperature were measured daily, whereas alkalinity, hardness, ammonia nitrogen and nitrite nitrogen were measured weekly, according to Strickland and Parsons (1972).

Major ions analysis

Samples of water from each tank were collected 1 d before the shrimp were stocked into the experimental tanks and then every 10 d during the 62-day rearing period. Water samples were analyzed for concentrations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ ions using an atomic absorption spectrophotometer (Hitachi 170-30, Japan), while Cl^- and SO_4^{2-}ion concentrations were analyzed using the titration method (UNEP GEMS,1994).

Statistical analysis

At the end of the 62-day rearing period, shrimp were harvested, counted and weighed. The percentage survival and growth rates of each treatment were recorded. Data from all experimental groups were analyzed statistically with the SPSS computer package for Windows (version 13.0). Differences were considered significant at the $P<0.05$ level.

RESULTS

Effects of PL_8 and PL_{10}, water salinity and stocking density on average final weight of *L. vannamei*

The average final weight, growth and survival rates of shrimp from all treatments after 62 days of culture are presented in Tables 1-4.

There were significant (P<0.05) differences in the average final weight of shrimp between postlarvae reared in water with salinity levels of 1 ppt and 3 ppt. The average final weight of treatments 1-6 (PL_8 and PL_{10}, 60-120 PL/m^2) reared in water with a low salinity level of 1 ppt was 2.89 g, while for treatments 7-12 (PL_8 and PL_{10}, 60-120 PL/m^2) reared in water with a low salinity level of 3 ppt, it was 2.16 g.

Treatments 1, 4, 7 and 10 (PL_8 and PL_{10}, 1 and 3 ppt, 60 PL/m^2), with the lowest stocking rate had the highest average final weight (2.84 g), whereas the highest stocking rate yielded the lowest average final weight (2.06 g). Moreover, the interaction between postlarval age and stocking rate, and the interaction between the salinity level of the water and stocking rate affected the average weight of shrimp. Treatments 6 and 12 (PL_{10}, 120 PL/m^2) had the highest average weight (3.16 g), while treatments 1 and 7 (PL_8, 60 PL/m^2) with the lowest stocking rate yielded the lowest average weight (1.75 g). There was a significant (P<0.05) difference in the average weight of shrimp between these groups.

Table 1 ANOVA for response of average final weight of *L. vannamei* reared in low salinity water levels of 1 ppt and 3ppt with three stocking densities.

Effect	Sum of squares	df	Mean squares	*F*-ratio	*P*-value [a]
PL	0.081	1	0.081	0.340	0.565
Salinity	4.445	1	4.445	18.614	0.000
Density	4.183	2	2.091	8.758	0.001
PL*salinity	0.535	1	0.535	2.242	0.147
PL*density	2.452	2	1.226	5.134	0.014
salinity*density	2.158	2	1.079	4.519	0.022
PL*salinity*density	0.651	2	0.326	1.363	0.275
Error	5.731	24	0.239		
Total error	251.835	36			
Total (corr.)	20.237	35			

R^2=0.717; R^2_{adj} = 0.587 (for df); df = degree of freedom.

[a] Significant at the 5% level.

Table 2 ANOVA for response of growth rates of *L. vannamei* reared in water with low salinity levels of 1 ppt and 3ppt with three stocking densities.

Effect	Sum of squares	df	Mean squares	*F*-ratio	*P*-value [a]
PL	5.38E-005	1	5.38E-005	0.804	0.379
Salinity	0.001	1	0.001	20.091	0.000
Density	0.001	2	0.001	7.622	0.003
PL*salinity	0.000	1	0.000	2.929	0.100
PL*density	0.001	2	0.000	5.308	0.012
salinity*density	0.001	2	0.000	5.418	0.011
PL*salinity*density	0.000	2	5.76E-005	0.861	0.436
Error	0.002	24	6.69E-005		
Total error	0.065	36			
Total (corr.)	0.006	35			

R^2=0.722; R^2_{adj} = 0.594 (for df); df = degree of freedom.

[a] Significant at the 5% level.

Effects of PL_8 and PL_{10}, water salinity and stocking density on growth of *L. vannamei*

At a salinity level of 3 ppt, treatments 1-6 (PL_8 and PL_{10}, 60-120 PL/m^2) had a growth rate of 0.047 g/d that was significantly (P<0.05) higher than in treatments 7-12 (PL_8 and PL_{10}, 60-120 PL/m^2) with a salinity level of 1 ppt, where the growth rate was 0.034g/d. Nevertheless, the survival rate of 80.9% for treatments 7-12 reared in water with the low salinity level of 3 ppt was higher than in treatments 1-6 that had a low salinity level of 1 ppt and a survival rate of 43.9%.

The highest growth rate of shrimp in treatments 1, 4, 7 and 10 (PL_8 and PL_{10}, 1 and 3 ppt), all at a stocking rate of 60 PL/m^2, was 0.046g/d, and no significant (P<0.05) difference of growth rate was observed with the stocking density at 90 PL/m^2. In contrast, the stocking density at 120 PL/m^2 had the lowest growth rate of shrimp (0.033g/d).

With the same salinity level in the water, there was a significant interaction between the stocking rate and the growth of shrimp, At a salinity level of 1 ppt and 90 PL/m^2, the growth

Table 3 ANOVA for response of survival rate of *L. vannamei* reared in water with low salinity levels of 1 ppt and 3ppt with three stocking densities.

Effect	Sum of squares	df	Mean squares	F-ratio	P-value [a]
PL	49.938	1	49.938	13.677	0.001
Salinity	12335.804	1	12335.804	3378.644	0.000
Density	2925.372	2	1462.686	400.614	0.000
PL*salinity	10.671	1	10.671	2.923	0.100
PL*density	7.507	2	3.754	1.028	0.373
salinity*density	313.191	2	156.595	42.890	0.000
PL*salinity*density	9.411	2	4.705	1.289	0.294
Error	87.627	24	3.651		
Total error	155914.880	36			
Total (corr.)	15739.520	35			

R^2=0.722; R^2_{adj} = 0.594 (for df); df = degree of freedom.

[a] Significant at the 5% level.

Table 4 Average weight, growth rate and survival rate of *Litopenaeus vannamei* reared in water with low salinity of 1 ppt and 3 ppt with three stocking densities.

Treatment	Average weight (g)	Growth rate (g/d)	Survival rate(%)
1	2.85±0.56	0.046±0.009	57.2±3.48
2	3.23±0.90	0.052±0.014	39.6±0.69
3	2.80±0.28	0.045±0.005	29.7±1.70
4	3.28±0.95	0.053±0.015	61.1±1.90
5	3.57±0.35	0.058±0.005	40.7±1.27
6	1.59±0.03	0.026±0.001	35.0±0.80
7	2.21±0.43	0.036±0.007	88.3±2.89
8	1.90±0.06	0.026±0.008	82.6±1.68
9	1.93±0.38	0.031±0.006	70.0±1.38
10	3.03±0.40	0.049±0.006	88.3±0.00
11	2.12±0.27	0.034±0.004	84.4±2.20
12	1.91±0.29	0.031±0.005	71.9±2.11

rate was 0.055 g/d, which was significantly (P<0.05) higher than in 1 ppt and 60 PL/m^2 which showed a growth rate of 0.049 g/d.

Effects of PL_8 and PL_{10}, water salinity and stocking density on survival of *L. vannamei*

At a salinity level of 3 ppt, treatments 7-12 (PL_8 and PL_{10}, 60-120 PL/m^2) had a survival rate of 80.9%, which was significantly (P<0.05) higher than for treatments 1-6, where the salinity level was 1 ppt and the survival rate was 43.9%. The treatments with the lowest stocking density (60 PL/m^2) had the highest survival rate of 73.7%. Treatments 1, 4, 7 and 10 (PL_8 and PL_{10}, 1 and 3 ppt), with stocking densities of 90 PL/m^2 and 120 PL/m^2, had a survival rate of 61.8% and 51.7 % respectively. There was a significant (P<0.05) difference in the survival rate of shrimp among treatments.

The highest survival rate of shrimp was 88.3% from treatments 9 and12 (3 ppt & stocking density 120 PL/m^2) while treatments 8 and 11 (3 ppt and stocking density 120 PL/m^2) had a survival rate of 83.5%, which was significantly (P<0.05) less.

Major ions and water quality analysis

The different ion concentrations during the 62-day culture period are presented in Table 5. The concentrations of the six major ions (Mg^{2+}, Ca^{2+}, Na^+, K^+, Cl^- and SO_4^{2-}) at a salinity level of 3 ppt (treatments 7-12) were significantly (P<0.05) higher than the salinity level of 1 ppt (treatments 1-6) throughout the experiment.

Water quality parameters during the 62 d of culture are presented in Table 6. Dissolved oxygen, temperature, pH, alkalinity, ammonia-nitrogen and nitrite-nitrogen from salinity levels of 1 ppt (treatments 1-6) and 3 ppt (treatments 7-12), respectively, were not significantly (P>0.05) different. Only hardness in the salinity level 3 ppt treatments was significantly (P>0.05) higher than those of 1 ppt treatments. Water quality parameters during the experiment remained within acceptable limits for *L. vannamei*.

DISCUSSION

Results from the 62-day trial in this study indicated that both PL_8 and PL_{10} exhibited better survival rates at a salinity level of 3 ppt than at 1 ppt. Similar results were reported by McGraw *et al*. (2002) and Davis *et al*. (2002), who demonstrated that during a 48-hour acclimation period, PL_{10} had greater survival rates at salinities in excess of 4 ppt than at salinities of 2 ppt and lower. McGraw and Scarpa (2004) found that PL age, salinity endpoint, and the rate of salinity reduction influenced PL survival following acclimation to a low salinity rearing environment. There were no significant differences in the survival rate of shrimp with either PL_8 or PL_{10} for stocking at the same density. The age range for tolerance to widely fluctuating salinity levels for most penaeid PL has been reported as between PL_{10} and PL_{40} (Mair, 1980; Cawthorne *et al*., 1983; Kumulu and Jones, 1995; Rosas *et al*., 1999; Tsuzuki *et al*., 2000). Based on the current data, it would appear that PL_8 and PL_{10} *L. vannamei* exhibited no significant difference in survival rates at low salinity levels ranging from 1 to 3 ppt.

The ionic profiles in this study were similar to full strength seawater, which for 1 ppt diluted seawater should have concentrations of various minerals as follows : Ca^{2+}, 11.6; Mg^{2+}, 39.1; K^+, 10.9; Na^+, 304.5; Cl^-, 551; and SO_4^{2-}, 78.3 mg/l, respectively (Davis *et al*., 2004). The ionic profiles of water from the treatments with a salinity level of 1 ppt (treatments 1-6) had higher concentrations of SO_4^{2-}, $Mg^{2+}$and K^+ when compared to seawater diluted to the same salinity. Other ions, such as Ca^{2+}, Na^+ and Cl^- , had concentrations similar to seawater. The growth rate of shrimp in treatments 1-6 was 0.047 g/d, which was the maximum growth rate in water with a normally low salinity of 1 ppt, if the ionic profiles

Table 5 Ion concentrations in water with low salinity levels of 1 ppt and 3 ppt during the 62 days rearing period of *Litopenaeus vannamei* with three different densities.

Treatment	Major ions (mg/L)					
	Cl^-	SO_4^{2-}	Ca^{2+}	Na^+	Mg^{2+}	K^+
1	636.5±127.6 [a]	132.56±10.90 [a]	23.39±7.59 [a]	325.60±81.37 [a]	54.03±4.76 [a]	18.80±0.84 [a]
2	622.2±106.2 [a]	130.23±9.35 [a]	24.97±6.93 [a]	305.72±78.78 [a]	53.22±3.77 [a]	18.41±0.37 [a]
3	611.2±98.2 [a]	130.94±6.00 [a]	22.81±6.80 [a]	287.17±59.63 [a]	51.63±3.91 [a]	18.36±0.39 [a]
4	627.6±104.0 [a]	129.59±8.04 [a]	24.50±7.18 [a]	316.90±81.20 [a]	53.42±3.72 [a]	18.09±0.61 [a]
5	637.5±87.3 [a]	128.53±10.73 [a]	25.25±7.40 [a]	309.14±61.79 [a]	52.87±3.12 [a]	18.33±0.52 [a]
6	622.0±88.0 [a]	128.53±13.01 [a]	20.16±6.72 [a]	305.36±65.54 [a]	48.25±11.48 [a]	18.38±0.92 [a]
7	1,524.1±402.0 [b]	246.98±6.66 [b]	49.25±19.66 [b]	801.28±220.73 [b]	131.03±17.06 [b]	36.45±3.77 [b]
8	1,545.7±361.1 [b]	255.02±13.43 [b]	57.57±26.86 [b]	785.19±239.00 [b]	130.03±18.54 [b]	37.29±3.73 [b]
9	1,509.3±351.0 [b]	262.53±10.60 [b]	54.41±25.41 [b]	753.60±212.44 [b]	128.22±19.04 [b]	37.02±4.13 [b]
10	1,489.0±362.1 [b]	266.07±10.45 [b]	51.40±22.70 [b]	755.12±171.17 [b]	129.13±17.45 [b]	36.45±4.52 [b]
11	1,548.8±346.8 [b]	260.08±12.55 [b]	50.70±24.26 [b]	751.00±185.63 [b]	128.60±18.09 [b]	36.57±3.41 [b]
12	1,550.8±378.6 [b]	248.74±10.33 [b]	50.05±25.23 [b]	750.42±210.91 [b]	128.62±20.01 [b]	36.46±3.91 [b]

Different lower case letters in the same column indicate a significant ($P<0.05$) difference. Values shown are mean±SD.

Table 6 Water quality parameters at low salinity levels of 1 ppt and 3 ppt during the 62 days rearing period of *Litopenaeus vannamei* with three different densities.

Treatment	Average of water quality parameters						
	Temperature (°C)	pH	DO (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Ammonia-Nitrogen (mg/L)	Nitrite-Nitrogen (mg/L)
1	28.1±1.0[a]	8.3±0.1[a]	6.7±0.3[a]	106.0±22.7[a]	334.7±46.0[a]	0.140±0.119[a]	0.101±0.046[a]
2	28.2±1.1[a]	8.2±0.1[a]	6.8±0.2[a]	102.4±25.8[a]	330.9±44.0[a]	0.153±0.154[a]	0.096±0.051[a]
3	28.2±1.0[a]	8.2±0.1[a]	6.7±0.2[a]	106.7±28.3[a]	337.0±50.3[a]	0.137±0.082[a]	0.096±0.050[a]
4	28.2±1.0[a]	8.3±0.1[a]	6.7±0.3[a]	107.6±27.7[a]	331.4±47.0[a]	0.143±0.125[a]	0.084±0.045[a]
5	28.2±1.0[a]	8.2±0.1[a]	6.8±0.2[a]	108.8±26.5[a]	336.2±51.3[a]	0.103±0.071[a]	0.109±0.056[a]
6	28.2±1.0[a]	8.3±0.1[a]	6.7±0.2[a]	107.4±28.5[a]	331.9±47.5[a]	0.150±0.095[a]	0.110±0.059[a]
7	28.2±1.0[a]	8.1±0.1[a]	6.7±0.2[a]	112.6±33.1[a]	597.4±144.9[b]	0.150±0.100[a]	0.114±0.018[a]
8	28.2±1.0[a]	8.2±0.1[a]	6.7±0.1[a]	114.3±31.0[a]	607.5±162.0[b]	0.163±0.080[a]	0.109±0.061[a]
9	28.2±1.0[a]	8.3±0.1[a]	6.7±0.3[a]	113.6±32.23[a]	586.2±138.2[b]	0.150±0.083[a]	0.101±0.047[a]
10	28.3±0.9[a]	8.2±0.1[a]	6.8±0.3[a]	114.7±30.6[a]	585.6±151.2[b]	0.200±0.061[a]	0.097±0.054[a]
11	28.3±1.0[a]	8.2±0.1[a]	6.8±0.3[a]	108.6±27.1[a]	575.0±132.2[b]	0.107±0.049[a]	0.111±0.062[a]
12	28.2±1.0[a]	8.3±0.1[a]	6.8±0.1[a]	113.1±30.7[a]	576.7±124.3[b]	0.120±0.061[a]	0.111±0.062[a]

Different lower case letters in the same column indicate a significant ($P<0.05$) difference. Values shown are mean±SD.

were similar to seawater diluted to 1 ppt.

The most important ions in osmoregulation are chloride and sodium (Castille and Lawrence, 1981; Ferraris *et al.*, 1986; Parado-Estepa *et al.*, 1987). The ionic composition of saline water appears to be more important than the salinity with regards to its effect on shrimp survival and growth. This is probably due to the fact that most of the water in this study contained adequate levels of Na^+ and Cl^- to meet the physiological requirements of shrimp. (It was possible that the salinity level of 3 ppt contained superior levels of all ions to meet the physiological and physiochemical needs of *L. vannamei*.). Since shrimp can obtain minerals from both ambient water and feed, dietary supplements of selected minerals could facilitate better survival and growth rates of shrimp cultured in low salinity conditions. However, the dietary mineral requirements of aquatic animals are strongly influenced by the level of minerals found in the water (Davis *et al.*, 1990). Davis *et al.* (2005) reported on the effects of $Mg^{2+}$and K^+ deficiencies on shrimp survival and growth. Low levels of $Mg^{2+}$and K^+ in saline water were responsible for PL mortality (Rahman *et al.*, 2005) and it was suggested that if the salinity were adequate, then Ca^{2+}, Mg^{2+} and K^+ were the three most important ions that determine the survival and growth of *P. monodon* in inland saline water. This makes sense, as the survival rates of both PL_8 and PL_{10} in the current study at a salinity level of 3 ppt were significantly higher than at 1 ppt. The concentrations of the six major ions at a salinity level of 3 ppt were about twofold in comparison to 1 ppt. These differences in the ionic concentrations affected mainly survival and the number of shrimp after the 62 days of rearing period of the study. Similar results were reported by Uysuwan (2008) and Limhang (2005) in a study using *L. vannamei*, as well as by Pattarakulchai *et al.* (2007) working with *P. monodon*. It should be noted that most small-scale shrimp farmers in Thailand add freshwater during the grow-out period to replenish losses resulting from evaporation and seepage, so that the salinity is diluted, particularly during the latter stages before harvesting. These practices affected the survival and growth rates of shrimp (Pattarakulchai *et al.*, 2007). However, growth rates in the current study were correlated to salinity levels and stocking rates in other treatment groups. Treatments with lower survival rates of shrimp also had higher growth rates than treatments with higher survival rates of shrimp at the completion of the 62-day feeding trial.

CONCLUSION

A 62-day feeding trial was conducted to determine the effects of PL ages and stocking densities on survival and growth rates of *L. vannamei* in water with low salinity levels of 1 and 3 ppt under laboratory conditions. The results showed that at 3 ppt, the survival rates of shrimp of both PL_8 and PL_{10} were significantly higher than those with a salinity level of 1 ppt in the treatment groups with the same stocking density rates and all six major ions concentrations. The treatment groups with the lowest stocking density had the highest survival rates. The high survival rate of shrimp affected significantly the growth of the shrimp at salinity levels of 1 and 3 ppt. Based on the results of the present study, it could be concluded that in order to achieve good survival and growth rates for *L. vannamei*, with the salinity level at 1 ppt, PL_8-PL_{10} should be stocked at density rates not exceeding 60 PL/m^2. For a salinity level of 3 ppt, PL_8-PL_{10} should be stocked between 60 and 90 PL/m^2. However, a consistent salinity level should be maintained throughout the culture period to attain good growth and survival rates. If freshwater needs to be added during the culture period to maintain the water level, the stocking density rate ought to be reduced as a consequence.

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