

Investigation of the Effects of Salinity and Dietary Protein Level on Growth and Survival of Pacific White Shrimp, *Litopenaeus vannamei*

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Abstract

It is presumed that in hypo- and hypersaline environments, shrimp's requirements for some specific nutrients, such as protein, may differ from those known in the marine habitat; however, few investigations have been conducted in this area of study. In the present investigation, the effects of salinity and dietary protein level on the biological performance, tissue protein, and water content of Pacific white shrimp, *Litopenaeus vannamei*, were evaluated. In a 3 × 4 factorial experiment, juvenile shrimp with an average initial weight of 0.36 ± 0.02 g were exposed for 32 d to salinities of 2, 35, and 50 ppt and fed experimental diets with crude protein contents of 25, 30, 35, and 40%. A significant effect of salinity on growth of shrimp was detected, with the growth responses (final weight, weight gain) ranked in the order 2 ppt (3.87, 3.50 g) > 35 ppt (3.40, 3.04 g) > 50 ppt (2.84, 2.47 g). No effects of dietary protein level or an interaction between salinity and protein on growth of shrimp were observed under the experimental conditions of this study. Percent survival of shrimp fed the highest protein content (40%, survival of 74%) was, however, significantly lower than those of shrimp fed the other feeds (25, 30 and 35% protein, survival of 99, 91, and 94%, respectively), a result likely associated with the concentration of total ammonia nitrogen, which increased significantly at increasing protein levels. Final water content of whole shrimp was significantly lower in animals exposed to 50 ppt (70.8%) than in shrimp held at 2 (73.7%) and 35 ppt (72.3%). No effect of salinity, protein, or their interaction was observed on the protein content of whole shrimp. The results of the present study are in agreement with reports of superior and inferior growth of *L. vannamei* reared in hypo- and hypersaline environments, respectively, as compared to what is generally observed in seawater.

Penaeid shrimp culture in low-salinity environments has been practiced for several years in Asia (Flaherty et al. 2000). More recently, this practice was adopted in the Americas, including countries like the USA, Mexico, and Ecuador, among others (Jory et al. 2003). Some species like the Pacific white shrimp, *Litopenaeus vannamei*, can tolerate culture salinities as low as 1 ppt (Samocho et al. 2001), and it has been attempted to raise it at even lower salinities (Saoud et al. 2003). With the worldwide expansion of low-salinity shrimp culture, the need of

formulated feeds that meet the shrimp's nutritional requirements under a rather unusual culture environment has been made evident. Indeed, nutrition of shrimp reared at low salinity has caught the eye of researchers, who have shown improvements in growth and survival of shrimp through manipulations of nutrients within the formulated feeds (Gong et al. 2003, 2004a). Recently, various aspects of requirements for specific nutrients by shrimp reared at low salinity, such as protein, lipids, and minerals, have been discussed at symposia and conferences (Cuzón et al. 2004; Davis 2004; Fox and Siccardi 2004; Gong et al. 2004b; Perez-Velazquez and González-Félix 2004). Bearing

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in mind the salinity of the marine environment (34–36 ppt), one cannot but be astonished by the osmoregulatory capabilities of these organisms. Furthermore, in areas with high rates of evaporation of pond water, like the Coast of Hermosillo, which is in the middle of the desert of Sonora, Mexico, it is not uncommon for culture salinities to progressively increase up to 50 ppt or higher, especially toward the end of the culture season. Therefore, it also is of great interest to examine the nutritional needs of shrimp exposed to such hypersaline environments.

Dietary protein requirements of shrimp have been widely studied. However, the literature shows a large variation of the optimal dietary protein level, for example, from 15% to more than 36% for the Pacific white shrimp (Colvin and Brand 1977; Smith et al. 1985; Cousin et al. 1991; Aranyakananda and Lawrence 1993; Teichert-Coddington and Rodriguez 1995). Protein is the main and also the most expensive component of shrimp feeds (Aranyakananda and Lawrence 1993). In addition, experimental evidence has shown higher dietary protein requirements of shrimp held at salinities lower than that of natural seawater (Shiau 1998; Rosas et al. 2001), which has been explained in terms of greater energy expenditure associated with osmoregulation. It is possible that this phenomenon also holds true in hypersaline water because shrimp also face greater osmoregulatory activity under such environments. For these reasons, knowledge of requirements for protein by shrimp raised at extreme salinities is needed urgently. Such studies will aid in the refinement of shrimp feed formulations for culture under these conditions.

This paper examines the effects of the dietary protein level on the biological performance of *L. vannamei* reared at both low and high salinity.

Materials and Methods

A 32-d experiment was conducted at the Wet Laboratory of Aquaculture Nutrition and Biotechnology of the Kino Bay Experiment Station (KBES), University of Sonora at Kino Bay, Sonora, Mexico, to examine the effects of dietary protein level and salinity on growth, survival, and tissue protein content of *L. vannamei*.

Source of Shrimp and Salinity Acclimation

Pacific white shrimp postlarvae (PL) were obtained from the shrimp larviculture laboratory “Maricultura del Pacífico, S.A. de C.V.,” Kino Bay, Sonora, Mexico. They were maintained in seawater for 1 mo at the KBES in an outdoor 10-m³ fiberglass raceway and fed a commercial shrimp feed (Camaronina; Agribrands Purina®, Ciudad Obregon, Sonora, Mexico) with a protein content of 40%. The total amount of shrimp was then subdivided into three equal parts, which were transferred to three 250-L circular polyethylene tanks for salinity acclimation (at a rate of 1 ppt/h). Animals in one tank were acclimated to 2 ppt by adding deionized water, while brine, composed of artificial sea salt (Coral Life® Salt, Carson, CA, USA) dissolved in deionized water, was added to another tank for acclimation to 50 ppt. Shrimp in the third tank remained in seawater (35 ppt). The organisms were subsequently transferred to the experimental system for initiation of the study at a mean body weight of 0.36 ± 0.02 g.

Experimental System and Treatments

Two identical experimental culture systems, each with fifty 30-cm-diameter circular polyethylene tanks, were employed. The bottom area and capacity of each tank was 0.07 m² and 19 L, respectively. They were filled up with 17.3 L of water and provided with aeration. Clean water was maintained throughout the experiment by applying high rates of daily water exchange, ranging from 10% of the water volume during the first week to 80% at the end. Temperature was controlled with two ambient oil heaters (110 V, 1500 W, Model HO 301; Home Essentials, Troy, MN, USA) and one oscillating fan heater (110 V, 1500 W, EWT Glen Dimplex, Sonnenberg, Germany).

Salinities of 2, 35, and 50 ppt and levels of dietary crude protein of 25, 30, 35, and 40% were tested in a 3 × 4 factorial experiment. All three salinities in the experimental systems were obtained by dissolving artificial sea salt (Coral Life® Salt) in deionized water, while the various crude protein levels of feeds were achieved with the formulations shown in

Table 1. Each experimental treatment was randomly assigned to at least eight replicate tanks, stocking shrimp at a rate of four shrimp/tank (56 individuals/m²). Feeds were added to tanks as to provide moderate excess, applying two feedings each day, one at 8000 h and one at 1700 h. Excess feed was siphoned out of the tanks each morning, along with feces and exuviae.

Water Quality

Water temperature, salinity, and dissolved oxygen were measured daily using a multifunction oxygen meter (Model Y85; YSI, Yellow Springs, OH, USA). The concentrations of ammonia, nitrite, and nitrate were quantified weekly

using spectrophotometric methods adapted from Solarzano (1969) and Spotte (1979).

Protein Analysis

Crude nitrogen of shrimp at initiation and upon termination of the experiment, as well of experimental feeds, was analyzed via combustion by the Dumas method (Ebling 1968) with a Leco FP-528 nitrogen/protein determinator (Leco Corp., St. Joseph, MI, USA), calculating crude protein content as $N \times 6.25$. Three composite samples per treatment were analyzed, with each composite sample consisting of four individuals (whole shrimp), each one taken from a different tank. The samples were oven dried at

TABLE 1. *Ingredient composition (% dry weight) of experimental feeds for Litopenaeus vannamei reared at three salinities.*

Ingredient	Formulation protein level (%)			
	25	30	35	40
Menhaden fish meal ^a	15.625	18.750	21.875	25.000
Soybean meal ^b	24.800	30.600	36.500	42.400
Wheat starch ^c	31.655	22.840	13.925	4.910
Whole wheat ^c	20.000	20.000	20.000	20.000
Menhaden fish oil ^d	2.150	2.740	3.330	3.920
Vitamin premix ^e	2.000	2.000	2.000	2.000
Calcium phosphate monobasic ^c	2.500	1.800	1.100	0.500
Trace mineral premix ^f	0.500	0.500	0.500	0.500
Soy lecithin ^g	0.500	0.500	0.500	0.500
Cholesterol ^c	0.200	0.200	0.200	0.200
Vitamin C ^h	0.070	0.070	0.070	0.070
Composition				
Protein content (%) ⁱ	27.6 ± 0.2	32.4 ± 0.1	37.0 ± 0.2	42.1 ± 0.1
Moisture (%) ⁱ	5.4 ± 0.2	5.1 ± 0.1	5.4 ± 0.1	4.5 ± 0.0
Total lipid (%) ⁱ	4.0	5.0	6.0	7.0
Gross energy (kcal/kg feed) ^j	3889.7	3944.2	3994.7	4049.7
Protein/energy ratio (mg protein/kcal) ^j	51.5	63.4	75.3	86.5

^a Omega Protein Inc., Hammond, Louisiana, USA.

^b De-hulled solvent-extracted soybean meal, Southern Sates Cooperative Inc., Richmond, Virginia, USA.

^c United States Biochemical Corp., Cleveland, Ohio, USA.

^d Omega Protein Inc., Reedville, Virginia, USA.

^e g/kg premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D3 (400,000 IU/g) 0.002, DL-alpha-tocopheryl acetate (250 IU/g) 8.0, alpha-cellulose 865.266.

^f g/100 g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.

^g Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, Indiana, USA.

^h 250 mg/kg active C supplied by Stay C[®] (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.

ⁱ Determined values. Means ± SD of triplicate samples.

^j Estimated values.

60 C for 24 h, their water content was measured, and then they were pulverized with a mortar and pestle and subsequently submitted to nitrogen/protein determination.

Statistical Analysis

Using culture system as a blocking factor, initial weight, final weight, weight gain, survival (arcsine-transformed and arcsine-untransformed data are presented), shrimp tissue protein, and water content were analyzed by means of three-way ANOVA with a significance level of $P \leq 0.05$. Water quality data were analyzed by repeated measures ANOVA with a significance level of $P \leq 0.05$. In the absence of interactions, one-way ANOVA of single factors was employed. Tukey's honestly significant difference method was employed as the mean separation procedure. Statistical analyses were performed using Statistical Analysis System software (1999–2000, Software Release 8.1; SAS Institute Inc., Cary, NC, USA).

Results

Water Quality

No effect of culture system (used as a blocking factor) was observed for any of the water quality parameters. Overall mean salinities (\pm SD, range) were 2.1 ppt (\pm 0.0, 1.50–2.60), 35.2 ppt (\pm 0.1, 34.1–35.5), and 50.2 ppt (\pm 0.2, 47.8–51.0). Water temperature did not vary significantly among treatments, while significantly higher oxygen content in the water was detected throughout the study as salinity decreased, resulting in the following order, $2 > 35 > 50$ ppt (Table 2). Protein level had an effect on the concentration of ammonia, showing significantly higher values at increasing protein levels (Table 3). Also, significant variations in the ammonia concentrations were detected as time progressed, reaching its highest values on Wk 2 (Table 3). In the case of nitrite and nitrate, significant effects of salinity, protein, and their interaction were detected, with higher concentrations observed at increasing protein levels and in seawater, as compared to 2 and 50 ppt water. Additionally, significant variations in the concentrations of these compounds were detected as time progressed (Tables 4 and 5).

TABLE 2. Repeated measures ANOVA (RMANOVA) of dissolved oxygen and temperature levels^a of experimental culture systems with *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.

	Oxygen (mg/L)	Temperature (C)
Salinity (ppt)		
2	5.9 \pm 0.1	30.5 \pm 0.2
35	5.0 \pm 0.1	30.5 \pm 0.1
50	4.5 \pm 0.1	30.5 \pm 0.1
Protein (%)		
25	5.2 \pm 0.6	30.5 \pm 0.1
30	5.2 \pm 0.6	30.5 \pm 0.1
35	5.2 \pm 0.6	30.5 \pm 0.1
40	5.1 \pm 0.7	30.5 \pm 0.2
RMANOVA $P > F$		
Between-subjects effects		
Culture system	0.2963	0.3579
Salinity	<0.0001	0.2287
Protein	0.0786	0.2053
Salinity \times protein	0.0986	0.3464
Within-subjects effects (Wilks' lambda)		
Time	<0.0001	0.1634
Time \times salinity	<0.0001	0.1654
Time \times protein	<0.0001	0.2331
Time \times salinity \times protein	<0.0001	0.1693

^a Values shown are overall means \pm SD derived from 32 observations.

Shrimp Biological Performance

No effect of culture system and no interactive effects of culture system, salinity, or dietary protein level were observed on final weight, weight gain, or survival of shrimp. There was, however, a significant effect of salinity on growth and food conversion ratio (FCR) and of dietary protein level on survival. Shrimp reared at 2 ppt had significantly greater final weight and weight gain than animals kept at 35 or 50 ppt. Additional differences in final weight and weight gain were detected between these treatments, with the former treatment performing significantly better than the latter. In terms of survival, animals fed the dietary protein level of 40% showed significantly lower survival rate as compared to animals receiving the other treatments (Table 6).

Protein Analysis

Determined protein content values of experimental feeds were 27.6 ± 0.2 , 32.4 ± 0.1 ,

TABLE 3. Repeated measures ANOVA (RMANOVA) of $\text{NH}_3\text{-N}$ levels (mg/L) (means \pm SD) of experimental culture systems with *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.¹

	Time			
	Wk 1	Wk 2	Wk 3	Wk 4
Salinity (ppt)				
2	1.5 \pm 0.6	5.6 \pm 2.9	5.6 \pm 3.4	2.5 \pm 0.7
35	1.5 \pm 0.5	6.2 \pm 2.9	4.0 \pm 2.7	1.5 \pm 1.3
50	1.6 \pm 0.8	5.2 \pm 2.7	5.5 \pm 2.2	2.1 \pm 1.2
Protein (%)				
25	0.8 \pm 0.2 ^d	2.2 \pm 0.5 ^d	2.9 \pm 1.9 ^b	1.0 \pm 0.4 ^b
30	1.3 \pm 0.2 ^c	4.7 \pm 0.8 ^c	4.9 \pm 1.7 ^{ab}	2.2 \pm 1.1 ^a
35	1.8 \pm 0.3 ^b	7.0 \pm 1.2 ^b	5.4 \pm 3.2 ^{ab}	2.0 \pm 1.0 ^{ab}
40	2.3 \pm 0.2 ^a	8.8 \pm 1.8 ^a	6.7 \pm 3.2 ^a	2.9 \pm 1.1 ^a
RMANOVA $P > F$				
Between-subjects effects				
Culture system	0.2265			
Salinity	0.2176			
Protein	<0.0001			
Salinity \times protein	0.5312			
Within-subjects effects				
(Wilks' lambda)				
Time	<0.0001			
Time \times salinity	0.0462			
Time \times protein	<0.0001			
Time \times salinity \times protein	0.3409			

¹ Means of protein effects with different superscripts in the same column are significantly different ($P < 0.05$).

37.0 \pm 0.2, and 42.1 \pm 0.1% (Table 1). Mean initial protein content of whole shrimp was 69.7 \pm 0.7%. No significant effects of culture system, salinity, dietary protein, or their interaction on tissue protein content were detected at the end of the experiment (Table 7).

A significant effect of salinity on shrimp water content was observed, in which animals maintained at 50 ppt had significantly lower water content than shrimp kept at 2 and 35 ppt (Table 7). No effects of culture system, protein, or an interaction between factors were detected.

Discussion

Exposure of shrimp to either very low (2 ppt) or high (50 ppt) salinity affected their growth significantly. Shrimp maintained at 50 ppt had statistically lower final weight and weight gain than animals kept at seawater salinity (35 ppt). Depressed growth of shrimp at high salinity has been reported for this species (Bray et al. 1994), as well as for other penaeids like *Penaeus latissulcatus* (Sang and Fotedar 2004) and *Farfantepenaeus californiensis* (Villareal et al. 2003).

It has been pointed out that under hypersaline conditions, shrimp are forced to perform osmoregulation at high cost (Spaargaren 1975, 1976; Subramanian and Krishnamurthy 1986; Jiang et al. 2000), eventually leading to fewer resources allocated to growth, which is supported by the observations of Villareal et al. (2003), who reported higher metabolic rate, as measured by the rate of oxygen consumption, reduced growth, and assimilated dietary energy of *F. californiensis* exposed to 45 and 55 ppt water, as compared to shrimp maintained at 25 and 35 ppt. Rosas et al. (2001) also reported depressed growth of *L. vannamei* cultured at high salinity (40 versus 16 ppt). The authors observed significantly higher hemolymph ammonia concentration of animals held at high salinity (7.2 mg/L) and suggested this factor as possible causative. Overall, the preceding evidence and the results obtained in the present study clearly indicate the importance of avoiding high salinity in commercial shrimp farming.

On the other hand, shrimp maintained at 2 ppt had greater final weight and weight gain than the

TABLE 4. Repeated measures ANOVA (RMANOVA) of $\text{NO}_2\text{-N}$ levels (mg/L) (means \pm SD) of experimental culture systems with *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.¹

	Time			
	Wk 1	Wk 2	Wk 3	Wk 4
Salinity (ppt)				
2	0.000 \pm 0.002 ^b	0.013 \pm 0.006 ^b	0.013 \pm 0.011 ^b	0.007 \pm 0.001 ^b
35	0.009 \pm 0.012 ^a	0.185 \pm 0.097 ^a	0.686 \pm 0.917 ^a	0.353 \pm 0.408 ^a
50	0.002 \pm 0.003 ^b	0.059 \pm 0.054 ^B	0.102 \pm 0.107 ^b	0.089 \pm 0.087 ^b
Protein (%)				
25	0.001 \pm 0.002	0.062 \pm 0.068	0.062 \pm 0.093 ^b	0.046 \pm 0.056 ^b
30	0.009 \pm 0.014	0.089 \pm 0.083	0.107 \pm 0.110 ^b	0.088 \pm 0.094 ^{ab}
35	0.002 \pm 0.005	0.114 \pm 0.116	0.294 \pm 0.438 ^{ab}	0.293 \pm 0.422 ^a
40	0.002 \pm 0.004	0.079 \pm 0.119	0.606 \pm 1.074 ^a	0.170 \pm 0.327 ^{ab}
RMANOVA $P > F$				
Between-subjects effects				
Culture system	0.4378			
Salinity	<0.0001			
Protein	0.0009			
Salinity \times protein	0.0006			
Within-subjects effects (Wilks' lambda)				
Time	<0.0001			
Time \times salinity	<0.0001			
Time \times protein	0.0004			
Time \times salinity \times protein	0.0063			

¹ Means of salinity or protein effects with different superscripts in the same column are significantly different ($P < 0.05$).

organisms of the other two treatments, a result also reflected in their respective FCR values, with significantly lower FCR observed at 2 ppt, as compared to 35 and 50 ppt, and in turn, lower at 35 ppt than at 50 ppt. These results agree with reports of better growth of *L. vannamei* cultured in low-salinity water. In a 35-d study, Bray et al. (1994) found significantly greater growth of *L. vannamei* juveniles reared at 5 and 15 ppt (12.22–12.36 g) versus 25, 35, and 49 ppt (8.48–10.95 g). Although not working at the low end of salinity tolerance by this species, Rosas et al. (2001) also detected significantly higher final weight of *L. vannamei* reared for 30 d at 15 ppt (2.0–2.3 g) than at 40 ppt (1.5–1.9 g). However, contradictory data also are available, for example, in a set of three experiments carried out at salinities of 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, and 30 ppt, Laramore et al. (2001) found no survival of *L. vannamei* below 2 ppt and statistically better growth of shrimp held at 30 ppt than at 2 and 3 ppt after 18–40 d. Ponce-Palafox et al. (1997) reported a significant interaction between salinity and temperature on

growth of *L. vannamei* when held for 40 d at combinations of 20, 30, 35, 40, and 50 ppt of salinity and 20, 25, 30, and 35 C of temperature. Shrimp had significantly greater growth at salinities higher than 20 ppt and temperature between 25 and 35 C. Ogle et al. (1992) observed significantly lower survival of *L. vannamei* held for 30 d at 2 ppt in comparison to 16 ppt. In addition, Sowers et al. (2005) recently reported no effect of salinity on growth of *L. vannamei* cultured for 21 d, when contrasting 2 versus 20 ppt. Similarly, in a 70-d study, Samocha et al. (1998) did not find an effect of salinity (2, 4, and 8 ppt) on growth of the same species, although the salinity range employed was probably too narrow to detect significant differences.

From all the above, the effect of low salinity on growth of *L. vannamei* does not appear to be conclusive. Nevertheless, the examination of a key factor, age/size of the shrimp employed, may help to explain, at least in part, the inconsistencies of the data. It has been experimentally demonstrated that the younger the shrimp, the poorer their biological response will be to

TABLE 5. Repeated measures ANOVA (RMANOVA) of $\text{NO}_3\text{-N}$ levels (mg/L) (means \pm SD) of experimental culture systems with *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.¹

	Time			
	Wk 1	Wk 2	Wk 3	Wk 4
Salinity (ppt)				
2	0.185 \pm 0.191	0.194 \pm 0.108 ^b	0.085 \pm 0.229 ^b	0.042 \pm 0.047 ^b
35	0.133 \pm 0.049	0.579 \pm 0.387 ^a	1.793 \pm 2.536 ^a	0.939 \pm 1.086 ^a
50	0.159 \pm 0.155	0.116 \pm 0.140 ^b	0.132 \pm 0.207 ^b	0.251 \pm 0.230 ^b
Protein (%)				
25	0.128 \pm 0.139	0.260 \pm 0.200	0.207 \pm 0.335 ^b	0.112 \pm 0.123
30	0.229 \pm 0.210	0.347 \pm 0.332	0.197 \pm 0.319 ^b	0.233 \pm 0.226
35	0.156 \pm 0.104	0.296 \pm 0.339	0.674 \pm 1.138 ^{ab}	0.814 \pm 1.126
40	0.123 \pm 0.079	0.282 \pm 0.404	1.601 \pm 2.972 ^a	0.483 \pm 0.835
RMANOVA $P > F$				
Between-subjects effects				
Culture system	0.3857			
Salinity	<0.0001			
Protein	0.0031			
Salinity \times protein	0.0006			
Within-subjects effects				
(Wilks' lambda)				
Time	0.0001			
Time \times salinity	<0.0001			
Time \times protein	0.0158			
Time \times salinity \times protein	0.0440			

¹ Means of salinity or protein effects with different superscripts in the same column are significantly different ($P < 0.05$).

low-salinity water, as indicated by the results of Davis et al. (2002), who observed poor growth and survival of *L. vannamei* PL younger than 15 d of age (PL15) in seawater diluted to or lower than 4 ppt. The authors also observed better performance of PL20 at salinities as low as 1 ppt, although not without the occurrence of high mortality rates (12–17% mortality after only 48 h of acclimation). This is consistent with the data discussed so far because in those instances of inferior growth and/or survival of shrimp reared at low salinity, rather young animals have been employed. For example, Ponce Palafox et al. (1997) used PL18 (initial weight of 0.01 g); Ogle et al. (1992) employed PL22, while in the set of experiments conducted by Laramore et al. (2001), PL30 (0.1 g) were used. Conversely, juvenile shrimp and not PL have been employed in the examples in which superior performance of shrimp held at low salinity has been found, for example, Bray et al. (1994) and Rosas et al. (2001) employed juveniles with initial weights of 1.63–2.17 and 0.36 g (initial weight of 0.36 g also for this study), respectively. Furthermore, Laramore et al. (2001) performed

a fourth experiment with three sizes of shrimp (PL25, PL40, and juveniles of 8 g), in which after 40 d of culture at a salinity of 2 ppt, survival of PL was only 14–29%, while juvenile shrimp had 100% survival. All these examples illustrate that growth and survival of *L. vannamei* are, to a significant extent, determined by an interaction between shrimp age/size and salinity and that the poor performance of shrimp observed at low salinity reported in the literature was possibly associated with the use of animals too young to withstand the osmoregulatory challenges of such environment.

The source of freshwater employed to adjust experimental salinities of the various studies is worth of some consideration. Except for the experiment of the present paper, none of the previous studies employed deionized or distilled water to adjust the experimental salinities. Instead, municipal tap water was generally used to either dilute seawater or to dissolve artificial sea salt (Ponce-Palafox et al. 1997; Sowers et al. 2005). Laramore et al. (2001) mixed well freshwater with well saltwater, while Rosas et al. (2001) did not specify their freshwater

TABLE 6. Initial weight, final weight, weight gain, survival, and FCR (means ± SD) of *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.¹

	Initial weight (g)	Final weight (g)	Weight gain (g)	Survival (%)	FCR
Treatment, salinity (ppt)/protein (%)					
2/25	0.35 ± 0.02	3.80 ± 0.28	3.44 ± 0.27	100.0 ± 0.0	2.2 ± 0.2
2/30	0.35 ± 0.02	3.94 ± 0.27	3.58 ± 0.27	94.4 ± 11.0	2.2 ± 0.2
2/35	0.36 ± 0.02	3.75 ± 0.46	3.39 ± 0.45	97.2 ± 8.3	2.3 ± 0.3
2/40	0.36 ± 0.02	3.98 ± 0.33	3.62 ± 0.34	80.5 ± 32.5	2.1 ± 0.2
35/25	0.36 ± 0.01	3.21 ± 0.27	2.85 ± 0.26	96.8 ± 8.8	2.7 ± 0.3
35/30	0.35 ± 0.02	3.61 ± 0.33	3.26 ± 0.32	81.2 ± 34.7	2.4 ± 0.2
35/35	0.36 ± 0.01	3.49 ± 0.36	3.12 ± 0.36	87.5 ± 18.8	2.5 ± 0.3
35/40	0.35 ± 0.01	3.30 ± 0.25	2.94 ± 0.27	84.3 ± 35.1	2.6 ± 0.2
50/25	0.35 ± 0.01	2.88 ± 0.39	2.53 ± 0.38	100.0 ± 0.0	3.1 ± 0.4
50/30	0.36 ± 0.01	2.80 ± 0.48	2.44 ± 0.47	96.8 ± 8.8	3.3 ± 0.9
50/35	0.37 ± 0.01	2.91 ± 0.58	2.54 ± 0.58	96.8 ± 8.8	3.2 ± 0.8
50/40	0.36 ± 0.01	2.72 ± 0.50	2.35 ± 0.49	56.2 ± 47.7	3.4 ± 0.7
Salinity (main effects)					
2	0.36 ± 0.02	3.87 ± 0.34 ^a	3.50 ± 0.34 ^a	93.0 ± 18.5	2.2 ± 0.2 ^a
35	0.36 ± 0.02	3.40 ± 0.33 ^b	3.04 ± 0.33 ^b	87.5 ± 26.1	2.6 ± 0.3 ^b
50	0.36 ± 0.01	2.84 ± 0.47 ^c	2.47 ± 0.46 ^c	87.5 ± 29.7	3.2 ± 0.7 ^c
Protein (main effects)					
25	0.35 ± 0.02	3.32 ± 0.49	2.96 ± 0.49	99.0 ± 5.0 ^a	2.7 ± 0.5
30	0.35 ± 0.02	3.46 ± 0.61	3.10 ± 0.61	91.0 ± 21.5 ^a	2.6 ± 0.7
35	0.36 ± 0.01	3.40 ± 0.58	3.03 ± 0.58	94.0 ± 13.0 ^a	2.6 ± 0.6
40	0.36 ± 0.02	3.42 ± 0.61	3.06 ± 0.62	74.0 ± 39.1 ^b	2.6 ± 0.6
ANOVA <i>P</i> > <i>F</i>					
Culture system	0.7546	0.2019	0.2128	0.8550	0.3523
Salinity	0.7280	<0.0001	<0.0001	0.6157	<0.0001
Protein	0.3631	0.3735	0.3748	0.0016	0.8972
Salinity × protein	0.8029	0.2606	0.2386	0.2201	0.6510

FCR = food conversion ratio.

¹ Main effects means of final weight, weight gain, survival, and FCR with different superscripts in the same column are significantly different (*P* < 0.05).

source. Tap or well freshwater always contain minerals that can vary in composition and concentration from, for example, locality to locality or time of the year. Taking into account that for low-salinity shrimp culture the water ionic profile can be more important than the salinity itself (Saoud et al. 2003), the apparently simple fact of using tap or well water could be an obstacle to make safe inferences about the data obtained. Hence, the use of deionized or distilled water should be preferred when trying to ascertain effects attributable to salinity.

The significant effect of salinity observed on growth of shrimp in the present study, ranked in the order 2 > 35 > 50 ppt, coincided with a significant effect of this factor on the mean water oxygen content in the exact same order. These differences can be considered normal because the capacity of water to hold oxygen

is inversely related to salinity. However, although oxygen levels were above critical low concentrations documented for *L. vannamei* and other penaeid shrimp (Seidman and Lawrence 1985; Rosas et al. 1999), the fact remains that the availability of oxygen was greater for shrimp held at low salinity, as compared to shrimp at 35 and 50 ppt. From the well-documented effect of medium oxygen availability on metabolism of penaeid shrimp (Rosas et al. 1999), the constantly higher oxygen concentration found at 2 ppt as possible causative or contributor to the attainment of greater final size of low-salinity cultured shrimp is worth of further investigation.

The final water content of whole shrimp, significantly lower in animals exposed to 50 ppt than in shrimp at 2 and 35 ppt, is consistent with the observations by Sang and Fotedar (2004), who measured significantly lower growth and

TABLE 7. Protein analysis and water content of whole *Litopenaeus vannamei* reared at three salinities and fed four levels of dietary protein.¹

	Shrimp protein content (% dry weight) ²	Shrimp water content (%) ²
Treatment, salinity (ppt)/protein (%)		
Baseline	69.7 ± 0.7	72.6 ± 0.4
Salinity (ppt) (main effects)		
2	74.9 ± 0.8	73.7 ± 0.9 ^a
35	74.8 ± 0.4	72.3 ± 1.9 ^a
50	74.5 ± 0.7	70.8 ± 1.8 ^b
Protein (%) (main effects)		
25	74.8 ± 0.9	71.7 ± 2.9
30	74.9 ± 0.7	72.1 ± 0.9
35	74.8 ± 0.6	72.4 ± 1.2
40	74.4 ± 0.6	72.8 ± 2.3
ANOVA $P > F$		
Culture system	0.3697	0.2954
Salinity	0.4306	0.0014
Protein	0.4289	0.5806
Salinity × protein	0.3089	0.3278

¹ Shrimp water content means of salinity main effects with different superscripts in the same column are significantly different ($P < 0.05$).

² Values are means ± SD of triplicate samples per treatment.

significantly diminished tail muscle water content of *P. latissulcatus* maintained at 46 ppt, as compared to shrimp held at salinities of 10, 22, and 34 ppt. The lack of differences observed between the 2 and 35 ppt treatments suggests effective acclimation to the low salinity. In a 60-d study with *Litopenaeus setiferus*, Rosas et al. (1999), measured significantly higher body water content of shrimp maintained at 15 ppt (74–75%) than at 35 ppt (67–67%), which indicates a less efficient capacity to overcome the water influx forces imposed by the low salinity, as compared to *L. vannamei*.

No effects of salinity, protein, or their interaction were observed on the protein content of whole shrimp. In this respect, Fang et al. (1992) reported no differences in the muscle amino acid composition of *Penaeus monodon* reared at salinities of 15, 30, and 45 ppt.

The lack of an effect of protein level observed on body protein content, as well as on growth of shrimp, may be attributed to the duration of the study (32 d), which may have not been long enough to detect significant differences. There

was, however, a significant effect of protein level on survival rate. Percent survival of shrimp receiving the feed with the highest protein content (40% protein, 74% survival) was significantly lower than those of shrimp fed the other feeds (25, 30, and 35% protein; 99, 91, and 94% survival, respectively). Because total ammonia nitrogen reached its highest mean levels also in tanks receiving this treatment, the result is likely associated with ammonia toxicity, taking into account that proposed critical total ammonia nitrogen levels range from 2.60 to 3.95 mg/L (Jiang et al. 1999; Lin and Chen 2001). Nitrite-N levels, which ranged from 0.0 to 0.3 mg/L, were always below critical levels reported for *L. vannamei*, 6.1 mg/L for 15 ppt water according to Lin and Chen (2003), and 0.45 mg/L in seawater according to Gross et al. (2004). Nitrate-N levels also were below critical recommended levels for penaeid shrimp, 232 mg/L according to Tsai and Chen (2002). Thus, it is not likely that these compounds contributed to shrimp mortality.

Significant effects of salinity, protein, and their interaction were observed on the concentrations of nitrite and nitrate. Higher levels of these compounds coincided with higher dietary protein content but only in 35 ppt water. Some studies have shown considerably slower nitrification kinetics of recirculating culture systems using seawater, as compared to freshwater (Nijhof and Bovendeur 1990; Chen et al. 2006). However, it is difficult to relate our results to nitrification processes within the culture systems because they were not provided with a biofilter and had high rates of water exchange (up to 80% daily).

In conclusion, significant differences in growth of *L. vannamei* juveniles exposed to different salinities were detected, with the growth responses ranked in the order 2 > 35 > 50 ppt. These results are in agreement with reports of superior and inferior growth of *L. vannamei* reared in hypo- and hypersaline environments, respectively, as compared to what is generally observed in seawater. No effects of dietary protein level or an interaction between salinity and protein on growth of shrimp were observed under the experimental conditions of this study.

Percent survival of shrimp fed the highest protein content (40%) was, however, significantly lower than those of shrimp fed the other feeds (25–35%), a result likely associated with the concentration of total ammonia nitrogen, which increased significantly at increasing protein levels. Final water content of whole shrimp was significantly lower in animals exposed to 50 ppt than in shrimp held at 2 and 35 ppt. No effects of salinity, protein, or their interaction were observed on the protein content of whole shrimp. Significant effects of salinity, protein, and their interaction were observed on the concentrations of nitrite and nitrate. Higher levels of these compounds coincided with higher dietary protein content only in seawater.

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