

Low Salinity Rearing of the Pacific White Shrimp *Litopenaeus vannamei*: Acclimation, Survival and Growth of Postlarvae and Juveniles

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Abstract

Low salinity rearing of *Litopenaeus vannamei* requires the transfer of postlarvae (PL) from high salinity hatchery systems to low salinity conditions. In order to determine effective transfer methods, fifteen-day-old postlarvae (PL15) were acclimated from a salinity of 30 ppt to endpoint salinities of 1 and 5 ppt using single-step and gradual salinity reduction procedures. Higher survival rates were obtained with gradual acclimation than with single-step acclimation. Survival of PL15 acclimated to 5 ppt was better than that of PL15 acclimated to 1 ppt. Survival of PL15 and juvenile stages in various low salinity treatments ranging from 0–5 ppt was also compared. In the PL stage, survival rates were good (>85%) in salinities over 1.5 ppt. Tolerance to low salinities increased with age and survival rate of juveniles (65%) was higher than that of PL (2%) in freshwater. In our long-term growth trials of juvenile shrimp maintained in different salinities, optimal growth comparable to that in normal seawater was observed in animals reared in a salinity of 5 ppt, demonstrating that *L. vannamei* can be successfully cultured in low-salinity inland systems.

Discipline: Aquaculture

Additional key words: Pacific white shrimp, salinity tolerance

Introduction

Japan is one of the world's largest importers of shrimps and prawns, with the country's average annual consumption amounting to 300,000 tons. However, Japan's aquaculture production of shrimp is only 1,700 tons per year¹². The only shrimp species cultured in Japan is the kuruma prawn, *Marsupenaeus japonicus*, and its culture industry in Japan has shown a decline over the years, mainly due to high production costs and viral disease outbreaks¹⁶. In this context, we are undertaking a research project funded by the Japanese government entitled "Development of land-based recirculating aquaculture systems for the domestic production of whiteleg shrimp *Litopenaeus vannamei*," aimed at developing modes of shrimp culture that are cost-effective, have low impact on the environment, and are relatively risk-free. Although the main objective of this research is to further Japan's domes-

tic culture industry, the technologies developed under the project are also expected to have applications to developing regions, especially in Southeast Asia.

The Pacific white shrimp, *Litopenaeus vannamei*, is considered to be one of the best suited species for inland culture, because of its higher tolerance to low salinities compared to other species such as *L. setiferus*⁹, *L. stylirostris*²¹ and *P. chinensis*⁵. Previous studies have shown that it is possible to successfully grow *L. vannamei* in low salinity waters²⁰. However, survival in the early stages of rearing is largely dependent on acclimation to low salinity conditions¹⁵. Postlarvae (PL) purchased from commercial hatcheries are usually shipped at high salinities. In order to be able to successfully transfer and culture these PL in low salinities, the first step would therefore have to be to establish a protocol for salinity acclimation.

In order to determine the optimal conditions for rearing *L. vannamei* in low salinity waters, it is also necessary to understand its salinity tolerance limits and effect of

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age on low-salinity survival. Salinity tolerance has been shown to vary with age of shrimp in a number of penaeid species including *L. vannamei*⁶, *M. japonicus*⁴, *L. setiferus*¹⁸, and *L. stylirostris*²¹. Although penaeid species exhibit tolerance to a wide range of salinities, this does not imply that animals can grow well at all the salinities. Moreover, ionic composition of water, and calcium, potassium and magnesium ions in particular have been shown to be more important than salinity itself in low-salinity aquaculture⁶. It is therefore also necessary to understand the influence of salinity and hardness on growth and survival.

This paper summarizes work that we carried out to a) develop a suitable acclimation procedure for *L. vannamei* PL for use in our Indoor Shrimp Production System (ISPS) developed by IMT Co. and operated by Yukiguni Suisan Co. at Myoko, Niigata, b) determine the interaction between age of postlarvae and ambient salinity, and c) identify the optimum low salinity for grow-out of shrimp.

Materials and methods

The experimental animals, specific pathogen-free (SPF) *L. vannamei* postlarvae (PL), were obtained from a commercial hatchery, Molokai Seafarms in Hawaii. Freshwater mixed with artificial sea salts (SeaLife, Marine Technology Pvt. Ltd., Japan) was used to make up salt concentrations of the artificial seawater (ASW) used in our experiments. Animals were maintained in closed recirculating tanks with constant aeration and fed once a day with crumbled commercial shrimp feed (Higashi Maru, Japan; 48% protein). Water temperature in the experimental tanks was set at 28°C. Three separate sets of experiments were carried out.

1. Acclimation rate

The first set of experiments was conducted to determine the effects of rate of salinity reduction from 30 ppt to two low salinity endpoints, 5 ppt and 1 ppt on PL survival.

In the first trial, fifteen-day-old postlarvae (PL15) were subjected to fast acclimation in a single step from 30 ppt to 5 ppt and 1 ppt, respectively. Fifty postlarvae stocked for one week at 30 ppt were transferred in three replicates to 45 L rectangular glass tanks containing 1 L artificial seawater (ASW) at 30 ppt, and 29 L of freshwater was added in a single step to obtain an endpoint salinity of 1 ppt. In the case of single-step acclimation to 5 ppt, animals were transferred to 4.25 L of ASW at 30 ppt and 20.8 L of freshwater was added to obtain an endpoint salinity of 5 ppt.

In the second trial, PL were gradually acclimated to salinities of 5 ppt and 1 ppt over a period of 1, 3 and 7

days, respectively. For gradual acclimation to 1 ppt, 100 PL at stage 15 were transferred from a salinity of 30 ppt to 60 L glass tanks containing 1.71 L of ASW at 30 ppt. Salinity was reduced to 1 ppt in a final volume of 50 L by the addition of equal volumes of freshwater 3-4 times a day over a 1, 3 or 7-day period. For gradual acclimation to 5 ppt, 100 PL were transferred to 60 L tanks containing 8.3 L of ASW at 30 ppt, and salinity was reduced to 5 ppt in a final volume of 50 L by 3-4 additions of freshwater each day for 1, 3 or 7 days.

Surviving shrimp in each tank were counted 48 h after completion of acclimation, and survival rate for each treatment was calculated. Results were statistically analyzed using analysis of variance (ANOVA) to determine significant differences in survival between treatments.

2. Low salinity tolerance

The second set of experiments was performed to determine the lowest levels of salinity that animals could tolerate without acclimation at the late postlarval (PL20) and juvenile stages. For each trial, 50 twenty-day-old postlarvae (PL20; average body weight 0.02 g) or juveniles (average body weight 1.0 g) were directly transferred from 30 ppt to 12 L tanks containing ASW of 0, 0.5, 0.75, 1.5, 2, and 5 ppt salinities, respectively, in replicates of three. Animals were maintained at 28°C with gentle aeration for 10 days. Surviving animals in each treatment were counted at the end of the experiment and survival rates were compared. Significant differences among treatments were assessed using one-way ANOVA.

3. Long-term growth

The third set of experiments was undertaken to study long-term growth of PL/juvenile shrimp maintained for 12 weeks at low salinities but with hardness levels adjusted, and to compare these results to growth in normal seawater. The purpose of this experiment was to determine whether the current low salinity and high hardness system in use in the ISPS facility can achieve optimum growth of shrimp, and to determine if salinity and hardness levels can be reduced further without affecting growth rates. Hardness levels for each treatment were adjusted by adding appropriate amounts of MgCl₂, CaCl₂ and NaHCO₃ to ASW. Treatments were as follows: 1) 30 ppt, 6,600 ppm hardness (normal salinity and normal hardness); 2) 5 ppt, 1,400 ppm (ISPS pool water; low salinity and high hardness, containing 2,000 mg L⁻¹ MgCl₂ and 395 mg L⁻¹ CaCl₂); 3) 2 ppt, 1,300 ppm (low salinity and high hardness, containing 2,000 mg L⁻¹ MgCl₂ and 395 mg L⁻¹ CaCl₂); and 4) 1.5 ppt, 450 ppm (low salinity and low hardness, containing 667 mg L⁻¹ MgCl₂ and 132 mg L⁻¹ CaCl₂). At the onset of the trial, 100 PL (mean initial weight 0.014 g) stocked at 5 ppt

for one week after gradual acclimation were weighed and transferred to four recirculating systems consisting of four 60 L tanks filled with ASW at the salinities and hardness levels to be tested. Body weights and lengths of shrimp in each tank were recorded at 4-week intervals.

Results were analyzed using analysis of variance (ANOVA) and Tukey’s test to determine significant differences in growth under the different experimental conditions.

Results

1. Acclimation rate

Survival of PL15 acclimated to 5 ppt was significantly higher than that of PL15 acclimated to 1 ppt at all the acclimation rates tested. Moreover, survival rates were higher following gradual acclimation compared to single-step acclimation (Fig. 1). In single-step salinity reduction, the survival rate of PL acclimated to 5 ppt (53%) was significantly higher than that of PL acclimated to 1 ppt (16%; ANOVA: $F_{1,4} = 22.67, p < 0.01$). Postlarvae acclimated to 5 ppt in 1 day showed significantly higher survival (94%) than PL acclimated to 1 ppt in 1 day (63%; ANOVA: $F_{1,4} = 21.14, p < 0.05$). Thereafter, in PL acclimated to 5 ppt, survival after 3- and 7-day acclimation was the same as that after 1-day acclimation, being nearly 96% (Fig. 1). However, for 1 ppt acclimation, survival rates increased with increasing time of acclimation, but it took 7 days of acclimation to attain a survival rate of 80% (Fig. 1). This shows that acclimation to very low salinities can be

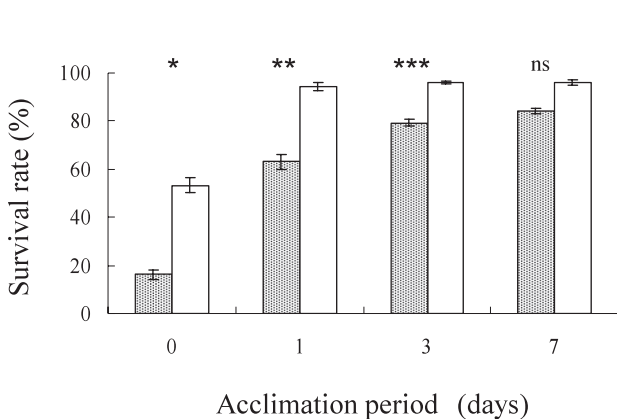


Fig. 1. Survival rates of *Litopenaeus vannamei* postlarvae (PL15) acclimated to salinities of 1 ppt and 5 ppt, using single-step (0 days) and gradual (1, 3 and 7 days) acclimation protocols

All data are presented as the mean ± SE. Significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ are indicated as *, ** and ***, respectively; ns indicates no significant difference.
 ■ 1ppt, □ 5ppt

achieved, but requires longer times than does acclimation to moderately low salinities.

2. Low salinity tolerance

Survival rates of PL20 and juveniles in different low salinities are shown in Fig. 2. The average survival rate of PL20 transferred to salinities of 1.5 ppt and above were seen to be high (> 85%), whereas survival in salinities of 0.75 ppt and 0.5 ppt was significantly lower at 47% and 29% (ANOVA: $F_{5,12} = 63.28, p < 0.001$), respectively. Survival rate in freshwater was only 2%. Tolerance to lower salinities was seen to increase with age, with juvenile shrimp exhibiting significantly higher survival in 0.75 ppt (93%; ANOVA: $F_{1,4} = 96.67, p < 0.001$), 0.5 ppt (77%; ANOVA: $F_{1,4} = 28.84, p < 0.01$) and 0 ppt (65%; ANOVA: $F_{1,4} = 114.49, p < 0.001$).

3. Long-term growth

In the long-term growth experiment, growth as evidenced by increase in body weight and body length of animals maintained in normal seawater (30 ppt, 6,600 ppm) and in ISPS pool water (5 ppt, 1,400 ppm) increased significantly over the 12-week trial period, compared to animals maintained in low salinity, high hardness (2 ppt, 1,300 ppm) and low salinity, low hardness (1.5 ppt, 450 ppm) treatments (Figs. 3a, b). Survival rates of the animals at the end of the trial were 86%, 96%, 46%, and 45% respectively, in normal seawater, ISPS pool water, low salinity and low hardness, and low salinity and high hardness treatments. Animals in the seawater and ISPS pool

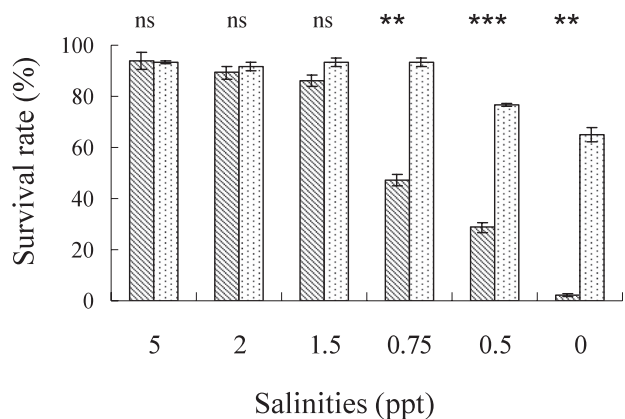


Fig. 2. Survival rates of *Litopenaeus vannamei* postlarvae and juveniles exposed to various low salinities without acclimation

All data are presented as the mean ± SE. Significant differences at $p < 0.01$ and $p < 0.001$ are indicated as ** and ***, respectively; ns indicates no significant difference.
 ■ PL20, □ Juvenile

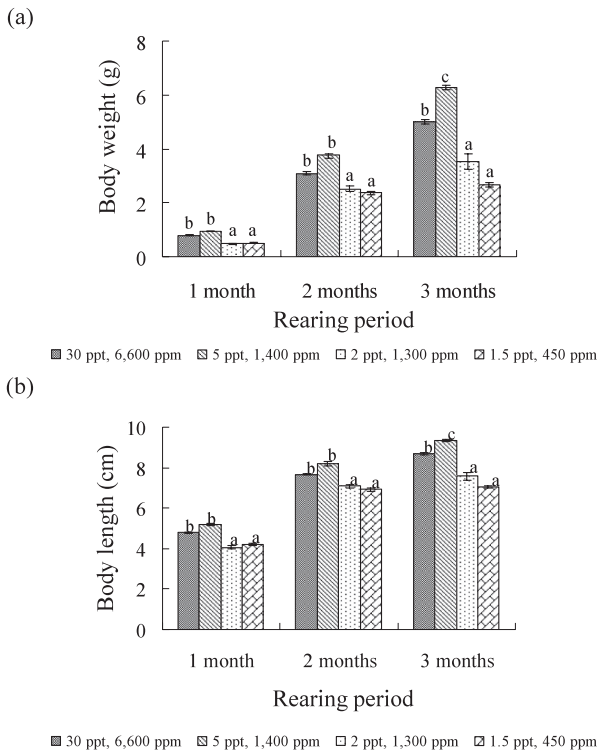


Fig. 3. Increase in (a) body weight and (b) body length, of *Litopenaeus vannamei* (starting at 0.014 g) reared in different salinities and hardness levels for three months

All data are presented as the mean ± SE. Significant differences at ($p < 0.05$) using Tukey's method are indicated by differing letters.

water treatments showed higher growth rates in terms of both body weight and length over the 3-month period. Further, in the latter phase, growth of animals in normal seawater and ISPS pool water was markedly higher than that of animals in the lower salinities. This indicates that *L. vannamei* culture in salinities lowered down to 5 ppt can achieve equivalent results as in seawater, as long as hardness is adjusted to appropriately high levels.

Discussion

L. vannamei postlarvae could be acclimated to a salinity of 5 ppt with a survival rate of nearly 100% using gradual acclimation procedures. The effects of endpoint salinity and acclimation rate on survival of *L. vannamei* PL were clearly demonstrated in the present study. PL 15 acclimated to an endpoint salinity of 5 ppt showed higher survival than those acclimated to 1 ppt, with gradual acclimation procedures producing significantly better results than single-step acclimation. Lengthening of acclimation time was observed to lead to increased survival in 1 ppt. This is in accordance with the findings of McGraw and

Scarpa¹⁴ who reported increased survival of PL15 following a 72-h acclimation period to 1 ppt. Van Wyk et al.²² have also demonstrated higher survival with extended acclimation periods at low salinities in *L. vannamei*. Abrupt transfer of *F. indicus* postlarvae from 30 ppt to 5 and 10 ppt without acclimation has been shown to cause mass mortalities¹⁰. Extended acclimation times are thought to better facilitate the equalization of ions between the shrimp hemolymph and the surrounding environment, thereby reducing osmoregulatory stress in the animal and leading to increased survival¹⁴. Although prolonged acclimation times can achieve increased survival in lower salinities, it is necessary to be able to acclimate PL to low salinities in the minimum time possible, for purposes of reducing costs. The results of our study indicate that an acclimation period of one day is sufficient for animals to be successfully acclimated to a salinity of 5 ppt; however, since longer acclimation times have been known to reduce osmoregulatory stress, an acclimation period of about 5 days was considered optimal to acclimate animals to a salinity of 5 ppt for grow-out in our ISPS system.

Our experiments on the effect of low salinity challenge on survival of *L. vannamei* at the PL and juvenile stages confirmed that both age and salinity significantly affected survival; survival rates increased as PL age increased and declined as salinity decreased. Juveniles could tolerate much lower salinities than PL. Similar results were also observed by Laramore et al.¹¹ in the same species. They reported better survival of juveniles in salinities of 2 ppt and 0 ppt, compared to postlarvae. Other studies in *L. vannamei*²⁰, and other penaeid species such as *M. japonicus*⁴, *P. indicus*¹⁰ and *L. setiferus*¹⁸ have also shown that salinity tolerance increases with PL age. Increased survival seen at the juvenile stages suggests that by this stage *L. vannamei* develops increased osmoregulatory capacity as has been reported for other decapod crustaceans⁴. PL size has been indicated to be indirectly correlated to a larger gill area, which in turn is thought to represent a better osmoregulatory capacity¹. From the results of our study, although PL20 could tolerate salinities as low as 2 ppt, highest survival of nearly 95% was achieved at 5 ppt, showing that it would be preferable to keep endpoint salinity at not less than 5 ppt in the ISPS system, in order to achieve maximum survival rates.

Our 12-week experimental trial on survival and growth of *L. vannamei* juveniles in low salinities showed that higher growth rates could be obtained at 5 ppt, 1,400 ppm as compared to 2 ppt, 1,300 ppm and 1.5 ppt, 450 ppm. There have been a few other studies on long-term growth and survival of this species in low salinities^{3,11,14,17}, but there are no reports on the effects of varying hardness levels in low salinity waters. However, some earlier stud-

ies have shown the importance of ions, especially Mg²⁺ and K⁺ in successful rearing of *L. vannamei* in low salinity waters^{7,19}. PL reared in low salinity well water at 4 ppt, supplemented with KCl and MgCl₂, was reported to show higher survival than animals reared in low salinity water without mineral supplements⁷. These results are in accordance with the results of our study, which also showed higher survival and growth rates in ISPS pool water (5 ppt, 1,400 ppm) containing higher levels of Mg²⁺ and K⁺. Laramore et al.¹¹ reported that size and salinity significantly affected growth in *L. vannamei*, and found growth at 2 ppt to be significantly lower than that at 30 ppt at the end of one month. However, they did not observe any significant differences in growth between 4 ppt and 30 ppt. In contrast to our results, Samocha et al.¹⁴ did not observe any difference in growth rates between animals maintained in 2 ppt and 4 ppt, although they did not compare growth in low salinities with that in 30 ppt. Similar to the results obtained in the present study, other studies on postlarvae and juveniles^{2,3} have also shown that growth of *L. vannamei* is slower at high salinity. This phenomenon has been attributed to the fact that in their natural habitat *L. vannamei* juveniles live in low-salinity lagoons for 6-12 weeks before returning to oceanic waters⁸. A salinity preference study conducted on *L. vannamei* postlarvae by Mair¹³ has also shown that they exhibit a strong preference for low salinity waters of 1-8 ppt. The results of the present study showed that culture of shrimp at a salinity of 5 ppt, with hardness increased to higher levels could yield growth rates comparable to, or even higher than that in normal seawater.

In conclusion, our study provided important information for adapting *L. vannamei* to culture in our indoor shrimp production system. The results obtained in this study have been successfully applied to acclimate and grow *L. vannamei* to marketable size at low salinities for the first time in Japan.

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