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**SHORT COMMUNICATION**

# EFFECT OF LOW SALINITY ON THE SURVIVAL OF POSTLARVAE OF THE BLUE SHRIMP, *LITOPENAEUS STYLIROSTRIS*, AT DIFFERENT STAGES

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## Abstract

The development of low salinity culture techniques is the major factor that facilitated the widespread cultivation of the white shrimp *Litopenaeus vannamei* in China. Similarly, adaptation to fresh or low salt water is critical for the commercial farming of a related species, the blue shrimp *L. stylirostris*. To determine the effects of low salinity on *L. stylirostris* development, the survival of postlarvae was investigated at different levels of salinity at the Haichang Jinhaian Shrimp Breeding Farm and Gulangyu Breeding Farm for Aquatic Animals of the Fisheries Research Institute of Fujian during 2002-2003. Results showed that both salinity and the age of the postlarvae significantly affected survival ($p$<0.01). The minimum seawater salinity for young postlarvae was 10-14 ppt and survival rates increased with age. When the post-larvae were 9 days old or older (≥P9), survival did not significantly differ in the low salinity treatments from that of the control (P12). The first hour was the most critical period. If larvae survived the first hour, they usually survived the whole study period (24 h) or longer.

## Introduction

The white shrimp *Litopenaeus vannamei*, fleshy shrimp *Penaeus chinensis,* and giant tiger prawn *Penaeus monodon* are the major cultured shrimp species in China. Since its introduction into China in the late 1980s, *L. vannamei* has become the leading cultivated species because it can be raised in waters of different salinities, including fresh, brackish, and sea water. The production of *L. vannamei* reached 66,025 tons in 2001, accounting for more than 75% of the total farmed shrimp production in China that year (Chinese

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Fisheries Bureau, 2002). Unfortunately, diseases such as the white spot syndrome virus (WSSV) in 1993 have reduced the annual production of farmed shrimp dramatically from more than 200,000 tons in 1991 to only 63,872 tons in 1994. Disease outbreaks have occurred more frequently in recent years, resulting in heavy losses for individual farmers. A solution is urgently needed, either by developing measures to control such diseases or by introducing disease-resistant species that can be cultivated in similar environments.

The blue shrimp *L. stylirostris* is one of the world's major farmed species. Although *L. stylirostris* has many characteristics similar to those of *L. vannamei,* the two species have major differences. Whereas *L. vannamei* grows well in low salinity environments, *L. stylirostris* spawns and survives in relatively high salinity (15-45 ppt; Mair 1980; Holtschmit et al., 1991; Samocha et al., 1998). *Litopenaeus stylirostris*, especially postlarvae (PL), is more sensitive to low salinity (0-5 ppt) than *L. vannamei* and can tolerate only a short period of no salinity without negative consequences to growth (Clifford III, 1998). Prolonged exposure to salinities less than 5 ppt result in death. On the other hand, *L. stylirostris,* especially juvenile and adult, is significantly more tolerant to low water temperatures (down to 15°C), enabling it to be cultured in the cold season (Lemair et al., 2002; Briggs et al., 2004), and more resistant to Taura virus infection than *L. vannamei* (Bray et al., 1994; Mohney, 1997). In general, *L. stylirostris* outperforms *L. vannamei* in low temperatures and high salinities (Robertson et al., 1991).

In China, there are vast inland areas with low salinity or fresh water that are suitable for shrimp farming. It is therefore important that any newly introduced species be able to grow in low salinity or fresh water. *Litopenaeus vannamei* is a good example; in recent years, it has become more tolerant to low salinity, leading to large-scale cultivation in China (Zeng et al., 2000;Yang et al., 2001; Zhu et al., 2002). Diseases, however, have emerged as a dominant threat to the shrimp farming industry. One potential solution is to find alternative species that are more resistant to various diseases. To determine the optimal salt concentration and test the salinity limits of *L. stylirostris*, we conducted two experiments cultivating *L. stylirostris* in water with various salt concentrations.

## Materials and Methods

To investigate the lethal low salinity for *L. stylirostris* postlarvae, we tested the survival rates of postlarvae of different stages in various salinity levels. Two experiments were carried out, one at the Haichang Jinhaian Shrimp Breeding Farm and one at the Gulangyu Breeding Farm for Aquatic Animals of the Fisheries Research Institute of Fujian, during 2002-2003. For each trial, 50 postlarvae of the tested stage (P2 to P12) were placed in each of eight 5-liter plastic containers with water of 26, 22, 18, 14, 10, 6, 3, or 2 ppt salinity. The remaining postlarvae were maintained in large ponds with a salinity of 27 ppt until they reached the next stage to be tested. Successive trials with larger postlarvae were conducted on successive days, as the postlarvae grew.

The experimental salinities were obtained by combining known amounts of fresh water and sea water and salt concentrations were measured with a hand salinity refractometer (Model S/Mill 6098, ATAGO Corp., Japan). We counted the surviving larvae and determined mortality one hour after placement in the various waters and every 6 hours thereafter for 24 hours. The water temperature in the experimental tanks fluctuated 25-31.0°C. As a control, P12 larvae were placed in water with a similar salinity. All shrimp were fed Artemia nauplii daily. Statistical analyses were processed and calculated with SPSS Software.

## Results

At 14 ppt or higher, the average survival rate at 24 h was at least 95% for the majority of the age groups (Table 1). In contrast, significant numbers of larvae younger than P6 died in salinities of 10 ppt or lower while significantly more P2 died in 14 ppt than in 18 ppt or high-

Table 1. Survival (no.) of various *Litopenaeus stylirostris* postlarvae at different salinities at 24 h[1].

| | *26 ppt* | *22 ppt* | *18 ppt* | *14 ppt* | *10 ppt* | *6 ppt* | *3 ppt* | *2 ppt* |
|---|---|---|---|---|---|---|---|---|
| P2 | 49.0 | 49.0 | 44.0 | **29.0** | 0.5 | 0.0 | 0.0 | 0.0 |
| P3 | 48.0 | 41.5 | 47.5 | 48.0 | **21.0** | 2.0 | 0.5 | 0.0 |
| P4 | 49.0 | 49.0 | 49.0 | 48.5 | 42.5 | 25.0 | 9.5 | 2.0 |
| P5 | 49.0 | 49.0 | 50.0 | 50.0 | 30.5 | 2.5 | 2.0 | 0.0 |
| P6 | 50.0 | 50.0 | 49.0 | 49.5 | 44.5 | **22.0** | 13.5 | 6.0 |
| P7 | 50.0 | 50.0 | 48.5 | 50.0 | 49.0 | 45.5 | 40.5 | 16.0 |
| P8 | 50.0 | 50.0 | 48.0 | 50.0 | 48.5 | 32.5 | **26.5** | 7.5 |
| P9 | 50.0 | 50.0 | 50.0 | 49.5 | 48.5 | 48.0 | 41.5 | **26.5** |
| P10 | 50.0 | 50.0 | 49.0 | 50.0 | 50.0 | 49.5 | 49.0 | 37.0 |
| P11 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 47.5 | 47.5 | 38.0 |
| P12 | 50.0 | 50.0 | 49.0 | 50.0 | 49.5 | 49.5 | 49.0 | 41.0 |
| Mean±SD | 49.6±0.7 | 49.0±2.5 | 48.6±2.0 | 47.7±6.2 | 39.5±15.8 | 29.5±20.0 | 25.4±20.5 | 15.8±16.5 |
| Survival (%) | 99.1 | 97.9 | 97.1 | 95.4 | 79.0 | 58.9 | 50.8 | 31.6 |
| *P*-value[2] | - | 0.957 | 0.663 | 0.094 | 0.000 | 0.000 | 0.000 | 0.000 |

[1] average of two experiments (n = 50 in each experiment)
[2] *P*-values were obtained using ANOVA with P12 in 26 ppt as control.

er. As the larvae increased in age, they became more tolerant of lower salt concentrations. For example, the majority of the P9 survived in water with salinity near the freshwater level. Except for P2, most PL survived the sudden reduction to 14 ppt while most PL younger than P9 died when transferred to 2 ppt. When the PL were ≥9 days old (P9 to P11), survival in low salinity (3 ppt or higher) did not significantly differ from that of the control (P12).

Two-way ANOVA analysis confirmed that both salinity and the age of the PL significantly affected survival ($p<0.01$), with the survival rate declining as salinity decreased and increasing as PL age increased (Table 2). In addition, the interaction of larval age and salinity played a significant role in survival.

For all larval ages, the first hour was the most critical period. If the larvae survived the first hour, then they usually survived the whole study period (24 hours), particularly for larvae younger than P8 (Fig. 1). For P8 and older, some were able to survive the first hour at 2 ppt, but died later.

## Discussion

Many studies have determined the effects of salinity on the survival of commercially important penaeid species such as *P. monodon* (Parado-Estepa, 1998), *P. japonicus* and *P. chinensis* (Charmantier-Daures et al., 1988), and *L. vannamei* (Mair, 1980; Bray et al., 1994; Atwood et al., 2003; Davis et al., 2004). Unlike these penaeid species, little information or experience on the freshwater cultivation of *L. stylirostris* is available in China, which limits the widespread cultivation of this species in areas without access to sea water.

Table 2. Effects of salinity and postlarvae (PL) stage, and the interaction between the two, on PL survival.

| *Source* | *Sum of squares* | *df* | *Mean square* | *F* | *Significance* |
|---|---|---|---|---|---|
| Salinity | 15.65 | 7 | 2.34 | 379.85 | 0.001 |
| PL stage | 14.10 | 10 | 1.41 | 239.67 | 0.001 |
| Salinity/PL stage | 16.14 | 70 | 0.23 | 39.19 | 0.001 |
| Error | 0.52 | 88 | 0.006 | - | - |
| Total | 46.41 | 175 | - | - | - |

Data were log-transformed before performing two-way ANOVA analysis with survival rate as the dependent variable.

This study provides important and practical information for adapting *L. stylirostris* to freshwater farming. Our results indicate that a salinity of 14 ppt or greater is critical for the survival of the most *L. stylirostris* PL (except P2) and that they can safely be stocked in water as low as 14 ppt without requiring an acclimation process. Based on this result, we recommend an initial salinity of 14 ppt or greater when beginning to acclimate *L. stylirostris* postlarvae. When salinity of the growing medium is 6 ppt or lower, the acclimation process, e.g., adding fresh water to the hatchery ponds, should proceed very slowly to avoid excess mortality.

Another finding of this study is that the age of *L. stylirostris* PL is a principle factor affecting the survival rate in low salinity. Our results suggest that P9 is the critical age for surviving in low salinity and that the ability of PL to live in a low salinity environment increases with PL age. These findings suggest that P9 or older can be stocked in low salinity without much effect on their survival.

According to our results and experience, acclimation may begin at stage P2, but is improved if begun with PL of later stages since viability increases with age. Postlarvae younger than 9 days are less tolerant to low salinity whereas the cut-off stage for *L. vannamei* is 4 days (Yang et al., 2001). When the *L. stylirostris* PL were 9 days or older, they tolerated salinity as low as 3 ppt without much mortality. This minimum salinity limit is consistent with findings of Charmantier et al. (1989) and Clifford III (1998) and similar to that of McGraw et al. (2002) for *L. vannamei*., implying that young *L. stylirostris* PL are more sensitive to low salinity than young *L. vannamei*. Thus, the early acclimation period should be carefully manipulated. When PL were placed in low salinity water, especially 14 ppt or less, the first hours constituted the key interval.

In commercial shrimp culture in China, a variety of methods have been used to adapt *L. vannamei* to low salinity. The simplest and the most frequently used method is gradual adaptation by slowly adding fresh water to nursery ponds as the PL age. But, in inland areas where sea water is unavailable, it is suggested that salt be added to freshwater ponds in which PL acclimate to the low salinity environment in which they are to be cultured. An extended observation carried out after the current experiment showed that *L. stylirostris* PL can adapt and grow normally in freshwater ponds to which a little salt had been added, and the adult shrimp reached commercial size with a survival rate greater than 90%. In approximately 100 days of culture, we harvested 33 kg commercial adult shrimps at 60-70 per kg (Yang et al., 2003) and concluded that *L. stylirostris* could be a promising species for freshwater cultivation for shrimp farmers in China in the near future.

Techniques of low salinity cultivation of *L.*

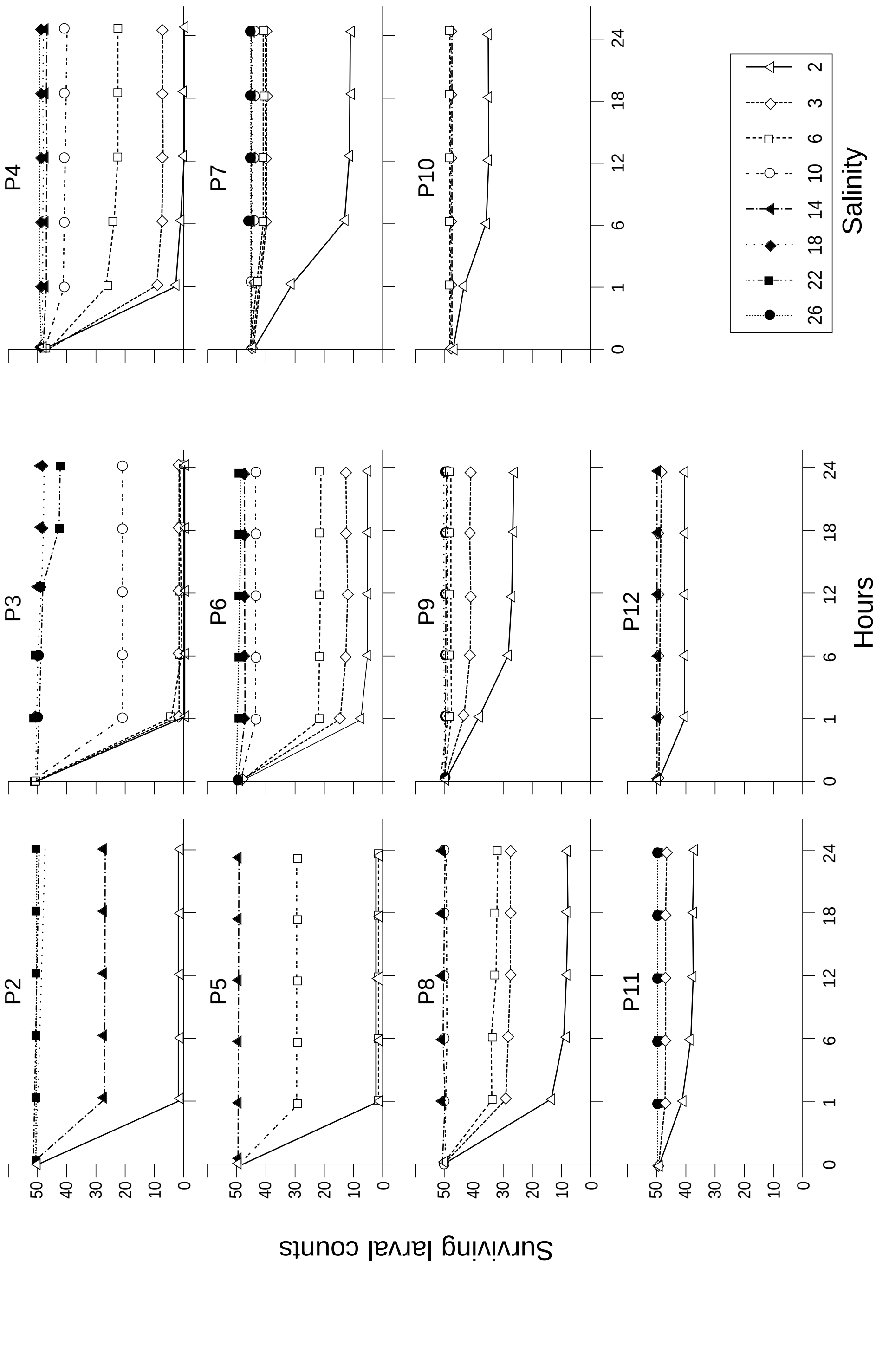


Fig. 1. Survival rates of postlarvae (PL) of different stages at various time points. Survival was averaged from two independent trials of 50 PL each.

*vannamei* have greatly benefited the common people of China since large-scale commercial shrimp culture has led to affordable consumer prices for average households since the late 1990s. Our experiment shows that *L. stylirostris* is also tolerant of a wide range of salinities and can survive and grow normally in low salinity environments. Compared to *P. vannamei*, *L. stylirostris* is more tolerant of low temperatures, enabling them to be cultured in cold areas of the northern part of China. Therefore, *L. stylirostris* is a potential species for cultivation in China in the future and research on the culture of *L. stylirostris* in low salinity water should be intensified.

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