Effects of artificial substrates on the growth, survival and spatial distribution of *Litopenaeus vannamei* in the intensive culture condition

Bo Zhang[1]; Wenhui Lin[2]; Jianrong Huang[1]; Yajun Wang[2]; Runlin Xu[1*]

Received: September 2009 Accepted: November 2009

Abstract

In order to investigate the effects of artificial substrates (vertical surface of polypropylene fabrics) on cultured shrimp, we reared 28-day old *Litopenaeus vannamei* post-larvae (PL28) intensively for 90 days at a density of 510 shrimp/m^2 in each of 8 tanks. Two tanks containing no artificial substrate were a control group, and 1, 3 and 5 artificial substrates were present in other 6 tanks. The volume of each tank was 100 L. In the tanks with artificial substrates, the percentage of shrimp distribution on the bottom was less significant ($P<0.05$) than that in the control tanks. The percentage of shrimps attached to the artificial substrates increased and fewer shrimp occupied the tank bottom as more artificial substrates were added to the tanks. Moreover the trends were more significant as rearing days increased. These results showed that artificial substrates could disperse the shrimp from the tank bottom onto the artificial substrates and thus alleviate the negative effect of high stocking density on shrimp growth in the tanks. Both the average weight and survival in the tanks with artificial substrates were significantly higher ($P<0.05$) than those in the control tanks. Furthermore, weight and survival increased when more artificial substrates were added. Because the shrimps in all tanks were supplied with suitable water quality and adequate nutritional food, we suggest that the differences of growth and survival were affected mainly by living space added with the addition of artificial substrates.

Keywords: **A**rtificial substrate, *Litopenaeus vannamei*, Spatial distribution, Survival, Weight

1- School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, PR China.

2- Pearl River Fisheries Research Institute, Guangzhou 510380, PR China.

*Corresponding author's email: lssxrl@tom.com

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

Introduction

White shrimp, *Litopenaeus vannamei*, has become one of the most important farmed shrimps in Central and South American countries, Thailand and China (Frias-Espericueta *et al*., 2001; McGraw *et al*., 2002; Saoud *et al*., 2003; Cheng *et al*., 2006). However, this industry has suffered drastic collapses from decreased growth and survival in over-crowded shrimp cultures. The reduction in growth and survival at higher densities results from a combination of factors, which include a decrease of favorable space and natural food sources, an increase in adverse shrimp behavior, such as cannibalism, a degradation of water quality and an accumulation of undesirable sediment (Kautsky *et al*., 2000; Arnold *et al*., 2006). In the shrimp culture industry, it is difficult to remove the uneaten feed and feces in ponds. Thus pond bottom pollution becomes more and more serious with increased rearing time. As benthic animals, however, shrimp are constrained to two-dimensional space rather than three-dimensional volume (Kumlu *et al*., 2001). As a result, shrimp that only live on the polluted bottom could easily be affected by several kinds of epizootic infectious disease resulting in high mortality (Lightner *et al*., 1987; Lightner, 1999; Saulnier *et al*., 2000; Yu & Song, 2000). Thus, finding a useful method to lessen the shrimp density effect at the pond bottom becomes an important concern. In some intensive production trials, artificial substrates such as fiberglass window screen, plastic mesh and commercial artificial substrates have been added to the shrimp culture system in an attempt to mitigate some of the negative effects of increased stocking density (Sandifer *et al*., 1987; Tidwell *et al*., 1998, 1999; Bratvold & Browdy, 2001). Studies indicate that artificial substrates could increase the natural food supplement for shrimp, improve the water quality of ponds, and control the pathogenic bacteria as biofilms (Thompson *et al*., 1999, 2002; Bratvold & Browdy, 2001; Burford *et al*., 2004; Preto *et al*., 2005; Arnold *et al*., 2005, 2006; Zarain-Herzberg *et al*., 2006; Ballester *et al*., 2007). Meanwhile, authors of some studies have speculated that the artificial substrates could lessen the negative effects by enlarging the living space for the shrimp, but obvious evidences were lacking (Tidwell *et al*., 1998, 1999; Kumlu *et al*., 2001; Arnold *et al*., 2005, 2006; Zarain-Herzberg *et al*., 2006). Furthermore, according to the previous research, which determined that growth and survival of *Farfantepenaeus paulensis* post-larvae were not enhanced in the presence of artificial substrates that had their biofilm periodically removed, the importance of using substrates for shrimp is not related to the space but to the availability of food provided by the biofilm formed on

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

the substrates (Ballester *et al*., 2003). So, a better understanding of the effect of artificial substrates on shrimp performance is necessary. In this study, the vertical surface of polypropylene fabrics was selected as the artificial substrate placed in the breeding tank. We tested the advantage of an artificial substrate for lessening the density effect by analysis of spatial distribution, survival and growth in intensive *L. vannamei* culture.

Materials and methods

Eight polyethylene tanks (50 cm diameter and 70 cm deep) were used to test four treatments (replicate): Group 0 (G0), as a control tank without artificial substrates, and Group 1 (G1), Group 3 (G3) and Group 5 (G5) with 1, 3 and 5 artificial substrates respectively. The structure of artificial substrates was based on the description of published research, which consisted of two modified polypropylene fabrics screens (50 × 20 cm, 0.2 cm thickness) with a large rough surface area and loose porous inner structure (Ballester *et al*., 2007). The substrates were fixed to a polyvinylchloride pole in the upper portion, which worked as a floater and to plumb ballasts in the bottom, to keep the screens vertically in the water column. All artificial substrates were placed in the water with a minimum of 10cm distance between them (G3 and G5). Each tank was filled with 100 L of water and the artificial substrates were immersed in the water. Twenty-eight-day-old *L. vannamei* post-larvae (PL28) (average weight 0.015 ± 0.003 g) obtained from a local commercial hatchery in Yangjiang City, Guangdong Province, China were stocked at a density of 100/tank (510PL/m^2) on June 12, 2008, and the shrimps were cultured for 90 days to September 10, 2008. Commercial shrimp feed (Haima^{TM}) was provided four times daily at 6:00, 12:00, 18:00 and 22:00. The amount of feed was adjusted daily for each tank based upon the amount of uneaten food observed. After 1.5 h of each feeding, we exchanged 10% culture water and filtered except during feeding time. Additionally, we supplied an underwater with continuous gentle aeration for each tank. Water quality parameters were measured twice a day (05:00 and 17:00). The following parameters were tested: pH with a portable pH meter (Hanna HI 991003), water temperature with a mercury thermometer; salinity with a hand-held refractometer (Optila HR 130), and dissolved oxygen (DO) with an oxygen meter (YSI model 58). The concentrations of ammonia and nitrite were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1993). To observe spatial distribution of shrimp in water, we took pictures from all treatments twice daily (10:00 and 16:00) by a digital camera (Canon IXUS 70)

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

which was fixed to a steel bar above tanks during the days of culture. We could take pictures when the camera was moved to the overhead of tank. According to pictures, shrimp distribution among the tanks was calculated including the percentage of shrimp distribution on artificial substrates (P1), on the tank bottom (P2), in the water column (P3) and on the tank wall (P4), respectively. In order to ease the analyzes, the mean percentage of every 10-day was compared among treatments. At the same time, the trial shrimp remaining in each tank were counted to determine survival rates. Shrimp average weights were obtained monthly by measuring 30 individual shrimps selected randomly from each tank. The shrimps were released in their original tanks after their weights were measured with sensitive balance Water quality data, the distribution percentage of shrimp attached on artificial substrates, shrimp weight, survival were analyzed by one-way ANOVA using SPSS 16.0 statistical software. Significant differences among the treatments were compared by LSD test. Differences were considered significant at the level of 0.05.

Results

Condition of water quality parameters during the course of the experiment were shown in Table 1. None of the monitored water quality parameters were significantly different among treatments during the days of culture. Each water quality parameter changed only slightly during the experimental period .The percentage distribution (%) among tank spaces including on the artificial substrates (P1), on the tank bottom (P2), in the water column (P3) and on the tank wall (P4) were shown in Figure 1. In the tanks without artificial substrates, the percentages distribution of P2 (59-64%), P3 (14-21%) and P4 (19-23%) remained relatively stable. However, in the tanks with artificial substrates, the percentage distribution of shrimp on P1 increased along the artificial substrates while declined on P2. In tank G5, when the percentage distribution of shrimp on P2 was the least (6%) at the end of the experiment, the percentage distribution on P1 reached the maximum (62%). There were no significant differences between the percentages distribution of shrimp of P4 (16-23%) and of P3 (13-21%) when we compared the experiment groups to the control group. The average weight of shrimp increased significantly as the number of artificial substrates in the tanks increased, and the average weight of shrimp obtained from all tanks with artificial substrates was significantly greater than the average weight of shrimp in the tanks without artificial substrates in 30 d, 60 d and 90 d (Table 2). After 90 days of culture, the average weight of the control group (5.22 g) was only 48.02% of the weight of the

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

experimental groups (10.87 g).In terms of survival; there was no significant difference among shrimp from all tanks.

Table 1: Water quality parameters during the course of the experiment

Treatments	WT (℃)	Salinity (‰)	pH	DO (mg l^{-1})	NH_3-N (mg l^{-1})	NO_2-N (mg l^{-1})
G0	25.8-27.4	26.7-27.2	7.2-7.4	7.8-8.2	0.16-0.21	0.08-0.13
G1	24.9-26.6	25.8-26.4	73.-7.7	7.5-8.5	0.19-0.23	0.06-0.11
G3	24.7-26.7	26.0-27.3	74.-7.7	7.5-8.6	0.15-0.21	0.03-0.10
G5	26.6-27.3	24.6-25.7	7.2-7.6	7.6-8.5	0.18-0.23	0.04-0.09

G0, G1, G3 and G5 denote the treatment with 0, 1, 3 and 5 artificial substrates, respectively

Table 2: Mean value (±SD for replicate) of weights (g) of *L. vannamei* reared from PL28 for 30 d, 60 d and 90 d; compared without artificial substrates (G0) and with 1 (G1), 3 (G3) and 5 (G5) artificial substrates

Treatment	Shrimp body weight (g)		
	30d	60d	90d
G0	1.07 ± 0.06^{a}	1.71 ± 0.08^{a}	5.22 ± 0.06^{a}
G1	1.71 ± 0.08^{b}	3.51 ± 0.02^{b}	8.26 ± 0.04^{b}
G3	2.02 ± 0.06^{c}	4.22 ± 0.06^{c}	10.70 ± 0.45^{c}
G5	2.31 ± 0.04^{d}	5.19 ± 0.06^{d}	13.65 ± 0.59^{d}

*Different superscripts denote significant differences ($P<0.05$) within cultivating days

within 30 d. From 40 d to 90 d, however, the survival of shrimp in all tanks with artificial substrates was significantly higher than in the tanks without artificial substrates, and the shrimp survival increased with the number of artificial substrates added (Figure 2). At the end of the experiment, the average survival rate of the control group was 54% while the average survival rate of the experimental groups reached 81%.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

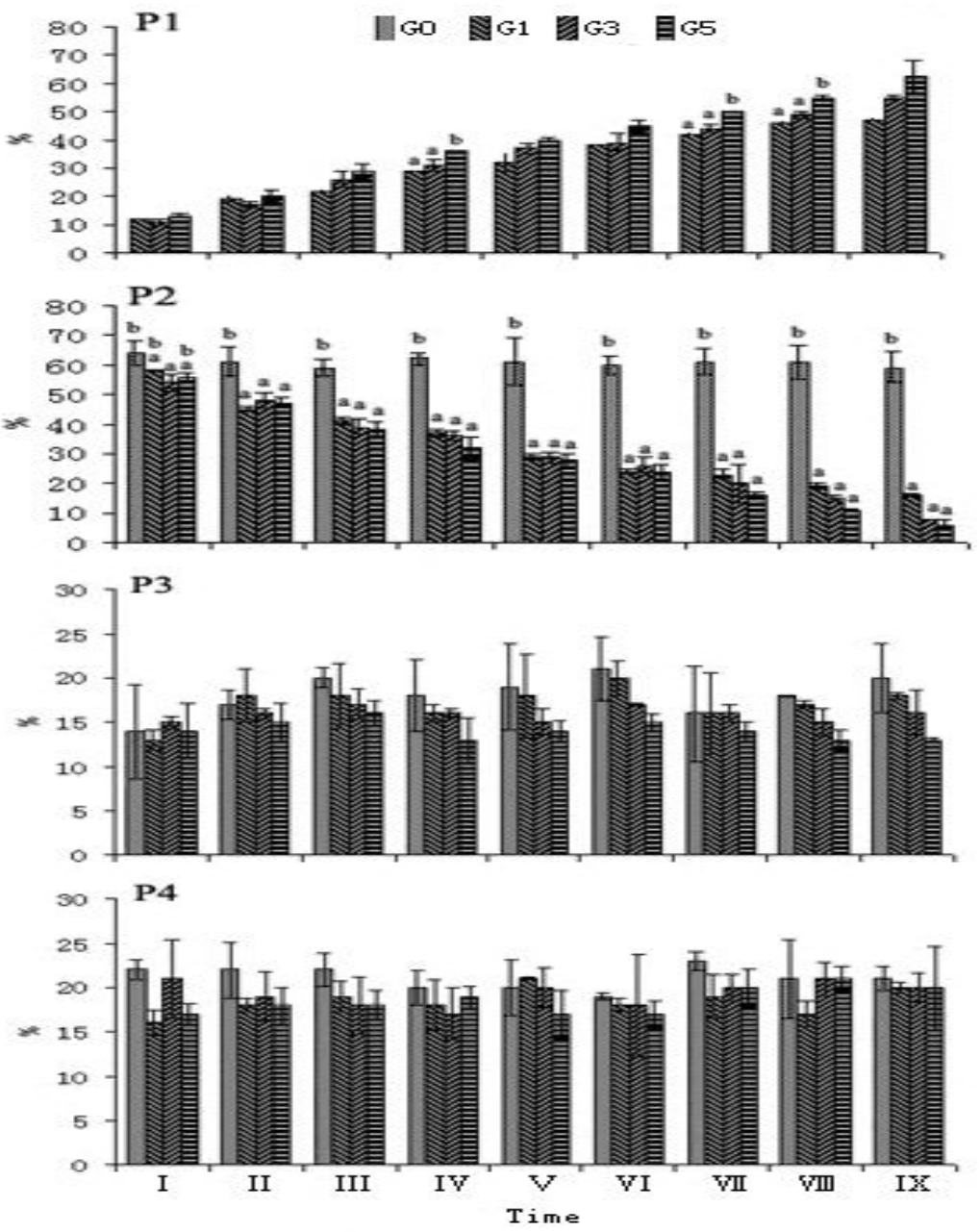


Figure 1: Mean value (±SD for replicate) of every10-day of distribution percentage (%) among tank spaces including on the artificial substrates (P1), on the tank bottom (P2), in the water column (P3) and on the tank wall (P4) of *L. vannamei* reared from PL28 for 90 days in tanks without artificial substrates (G0) and with 1 (G1), 3 (G3) and 5 (G5) artificial substrates. Different superscripts denote significant differences (P<0.05) within cultivating days

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

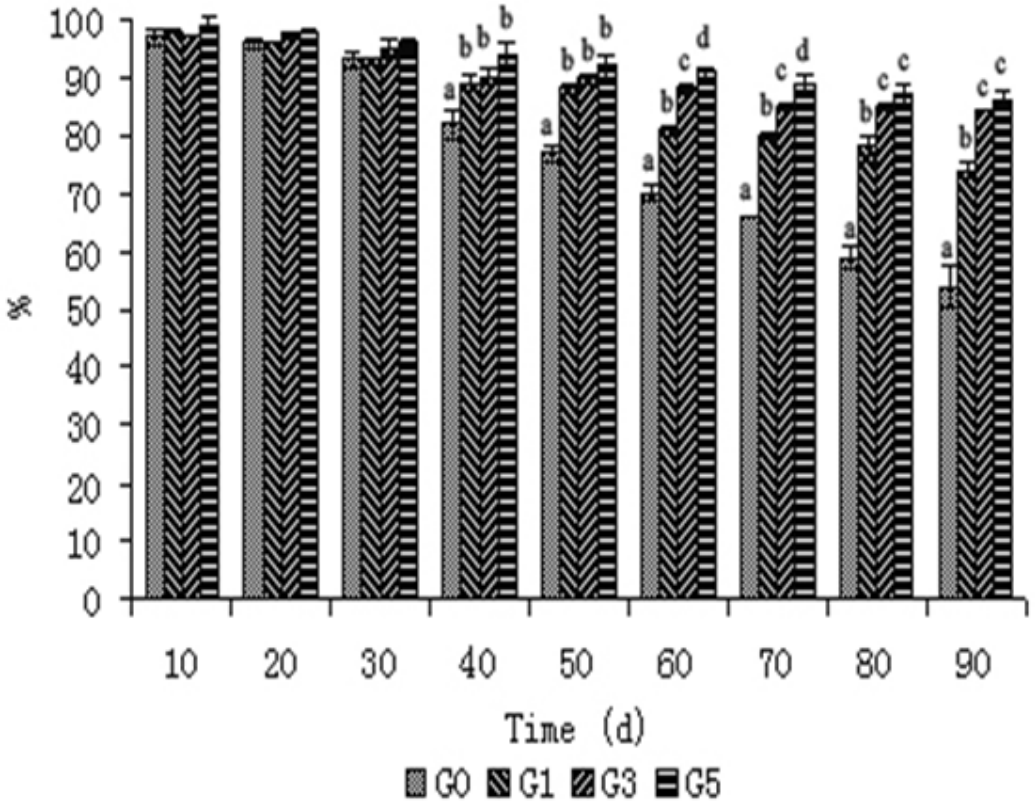


Figure 2: Mean value (±SD for replicate) of survival (%) of *L. vannamei* reared from PL28 for 90d, compared without artificial substrates (G0) and with 1 (G1), 3 (G3) and 5 (G5) artificial substrates. Different superscripts denote significant differences (P<0.05) within cultivating days

Discussion

In this study, modified polypropylene fabrics with a non-transparent loose porous inner structure were used as artificial substrates in a shrimp culture tank. This substrate could disperse the shrimps without hindering the exchange of water and matter in each tank. On the other hand, since the artificial substrates were placed vertically in the tanks, the shrimp distribution could be observed directly by camera, and the commercial feed could be dispersed to the tank bottom.

Though the shrimp were cultured intensively, no severe disease appeared during the experiment. The shrimp may have benefited from the good water quality management. Continuous filtering not only quickly removed the undesirable sediment but also maintained a slight water flow in tank, which was the similar to the natural environment for shrimp. Meanwhile, multiple-low-dose water exchange decreased the concentration of the harmful substances and maintained the

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

stable water quality parameters. Moreover, a continuous gentle aeration provided plenty of dissolved oxygen to avoid toxic matter. The known limiting factors in water quality such as DO, ammonia, nitrite, water temperature, pH, and salinity were within "safe" levels recommended for optimal growth and survival of penaeid shrimp (Chen & Lei, 1990; Chien, 1992).

During the experiment, in the tank without artificial substrates, most of the shrimps stay only on the tank bottom the entire time. However, the balance of the shrimp distribution percentage among tanks changed as soon as artificial substrates were placed in the tanks. The distribution percentage on the bottom of all tanks with artificial substrates was significantly lower than that without artificial substrates. With an increasing number of artificial substrates, more shrimp attached to artificial substrates, and fewer shrimp occupied tank bottom. Moreover, this difference became increasingly significant with a longer rearing time. It was suggested that artificial substrates could disperse the shrimp from tank bottoms to artificial substrates and thus alleviate the negative effect of the high density on shrimp production. This distribution may have resulted from the living habits of *L. vannamei*, which exhibited not only territorial and cannibal behavior but also little burrowing activity (Boddeke, 1983).

Many studies indicated that artificial substrates could improve the growth and survival of cultured shrimp and gave different explanation (Sandifer *et al*., 1987; Tidwell *et al*., 1998; Peterson & Griffith, 1999; Bratvold & Browdy, 2001; Moss & Moss, 2004; Arnold *et al*., 2006; Zarain-Herzberg *et al*., 2006; Ballester *et al*., 2007). The results of this study also indicated that shrimp growth and survival could increase with an increasing number of artificial substrates. In our study, shrimp were provided favorable water quality and commercial artificial feed with sufficient nutrition in all tanks. Therefore, the results of the present study suggested that the difference of the shrimp growth and survival in this study were affected mainly by living space added with the addition of artificial substrates.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

Acknowledgements

We are grateful to Mr. Liu Kailiang for providing facilities for carrying out the work. We would also like to thank Mr. Wu Linhui and Mr. Lin Yuanqiang for their cooperation during the experiment. This research has been financially supported by China National Department Public Benefit Research Foundation (No. nyhyzx07-043 and No. 200803013).

References

APHA, 1992. Standard methods for the examination of water and wastewaters, 18th ed. American Public Health Association, Washington, DC. 1268 pp.

Arnold, S.J., Sellars, M.J., Crocos, P.J. and Coman, G.J., 2005. Response of juvenile brown tiger shrimp (*Penaeus esculentus*) to intensive culture conditions in a flow through tank system with three-dimensional artificial substrate. Aquaculture. **246**:231-238.

Arnold, S.J., Sellars, M.J., Crocos, P.J. and Coman, G.J., 2006. Intensive production of juvenile tiger shrimp *Penaeus monodon*: An evaluation of stocking density and artificial substrates. Aquaculture. **261:**890-896.

Ballester, E.L.C., Waisielesky, W.J., Cavalli, R.O. and Abreu, P.C., 2007. Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: Biofilm composition and shrimp performance. Aquaculture. **269**:355-362.

Ballester, E.L.C., Wasielesky, W.J., Cavalli, R.O., Santos, M.H.S. and Abreu, P.C., 2003. Influência do biofilme no crescimento do camarão-rosa *Farfantepenaeus paulensis* em sistemas de berçário. Atlântica. **25:**117-122.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

Boddeke, R., 1983. Survival strategies of penaeid shrimps and their significance for shrimp culture. In: Proc. First Intl. Conf. Warmwater Aquaculture. (Eds. Roger, G.L., Day, R., Lim, A), pp: 514-523, Lpuisiana, USA.

Bratvold, D. and Browdy, C.L., 2001. Effects of sand sediment and vertical surfaces (Aquamats™) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture. **195**:81–94.

Burford, M.A., Sellars, M.J., Arnold, S.J., Keys, S.J., Crocos, P.J. and Preston, N.P., 2004. Contribution of the natural biota associated with substrates to the nutritional requirements of the post-larval shrimp, *Penaeus esculentus* (Haswell), in high-density rearing systems. Aquaculture Research. **35**:508-515.

Chen, J.C. and Lei, S.C., 1990. Toxicity of ammonia and nitrite to *Penaeus monodon* juveniles. Journal of the World Aquaculture Society. **21**:300-306.

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X. and Qi, X.J., 2006. Effects of dietary calcium, phosphorus and calcium / phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture. **251**:472-483.

Chien, Y.H., 1992. Water quality requirements and management for marine shrimp culture. In: Wyban J (Eds), Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society ,Baton Rouge, LA, USA, 30-42.

Frias-Espericueta, M.G., Voltolina, D. and Osuna-Lopez, J. I., 2001. Acute toxicity of cadmium, mercury, and lead to whiteleg shrimp (*Litopenaeus Vannemei*) postlarvae. Bulletin of Environmental Contamination and Toxicology. **67**:580-586.

Kautsky, N., Ronnback, P., Tedengren, M. and Troell, M., 2000. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture. **191:**145-161.

Kumlu, M., Eroldogan, O.T. and Saglamtimur, B., 2001. The effects of salinity and added substrates on growth and survival of *Metapenaeus monoceros* (Decapoda: Penaeidae) post-larvae. Aquaculture. **196**:177-188.

Lightner, D.V., 1999. The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture. **9**:27-52.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

Lightner, D.V., Hedrick, R.P., Fryer, J.L., Chen, S.N., Liao, I.C. and Kou, G.H., 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important disease. Fish Pathology. **22**:127-140.

McGraw, W.J., Davis, D.A., Teichert-Coddington, D. and Rouse, D.B., 2002. Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity, endpoint and rate of salinity reduction. Journal of the World Aquaculture Society. **33**:78-84.

Moss, K.R.K. and Moss, S.M., 2004. Effects of artificial substrate and stocking density on the nursery production of pacific white shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. **35**:537-542.

Peterson, J.J. and Griffith, D.R.W., 1999. Intensive nursery systems. Global Aquaculture Advocate. **2**:60-61.

Preto, A.L., Cavalli, R., Pissetti, T., Abreu, P.C. and Wasielesky, W.J., 2005. Efeito da densidade de estocagem sobre o biofilme e o desempenho de pós-larvas do camarão-rosa *Farfantepenaeus paulensis* cultivados em gaiolas. Cienc Rural. **35:**1417-1423.

Sandifer, P.A., Hopkins, J.S. and Stokes, A.D., 1987. Intensive culture potential of *Penaeus vannamei*. Journal of the World Aquaculture Society. **18**:94-100.

Saoud, I.P., Davis, D.A. and Rouse, D.B., 2003. Suitability studies of inland well waters for *Litopenaeus vannamei* culture. Aquaculture. **217**:373-383.

Saulnier, D., Haffner, P., Goarant, C., Levy, P. and Ansquer, D., 2000. Experimental infection models for shrimp vibriosis studies: review. Aquaculture. **191**:133-144

Thompson, F.L., Abreu, P.C. and Cavalli, R.O., 1999. The use of microorganisms as food source for *Penaeus paulensis* larvae. Aquaculture. **174**:139-153.

Thompson, F.L., Abreu, P.C. and Wasielesky, W.J., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture. **203**:263-278.

Tidwell, J.H., Coyle, D.C. and Schulmeiste, G., 1998. Effects of added substrate on the production and population characteristics of freshwater prawns *Macrobrachium rosenbergii* in ponds. Journal of the World Aquaculture Society. **29**:17-22.

Tidwell, J.H., Coyle, S., Weibel, C. and Evans, J., 1999. Effects and interactions of stocking density and added substrate on production and population structure of freshwater

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]

prawns *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society. **30**:174-179.

Yu, C.I. and Song, Y.L., 2000. Outbreaks of Taura syndrome in Pacific white shrimp (*Penaeus vannamei*) cultured in Taiwan. Fish Pathology. **35**:21-24.

Zarain-Herzberg, M., Campa-Córdova, A.I. and Cavalli, R.O., 2006. Biological viability of producing white shrimp *Litopenaeus vannamei* in seawater floating cages. Aquaculture. **259**:283-289.

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2010.9.2.9.3]