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# Effects of Artificial Substrates on Growth, Spatial Distribution and Non-Specific Immunity Factors of *Litopenaeus vannamei* in the Intensive Culture Condition

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**Abstract**

In order to investigate the effects of artificial substrates on shrimp growth, spatial distribution and non-specific immunity factors, such as phenoloxidase (PO), superoxide dismutase (SOD), peroxidase (POD), antibacterial activity (Ua), lysozyme (Ul) and hemolysin activity, 30-day old *Litopenaeus vannamei* post-larvae (PL30) were reared intensively for 60 days at a density of 500 shrimp $m^{-2}$ of water surface area in each of 8 PVC aquaria (1.0×1.0×1.5m, water surface area 1 $m^{-2}$, water volume 1000 L). Two aquaria containing no artificial substrate were a control group, and 1, 2 and 3 artificial substrates were present in other 6 aquaria. Shrimp growth (gain in weight), survival and yield increased significantly ($P<0.05$) when more artificial substrates were added. The percentage of shrimp on artificial substrates increased with increasing number of artificial substrates and rearing time. As for non-specific immunity factors, the activities of PO, Ua, Ul, POD, SOD and hemolysin of groups with artificial substrates were lower than those of control group significantly ($P<0.05$). With an increasing number of artificial substrates, the activities of PO, Ua, Ul, POD and hemolysin reduced, but SOD activity increased. It was suggested that artificial substrates could alleviate the negative effect of the high stocking density by providing well living environment for shrimp.

*Keywords*: White shrimp, high stocking density, living space

## 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). In China, many commercial farms stock 50-100 shrimp $m^{-2}$ (extensive to semi-intensive) to 200-400 shrimp $m^{-2}$ (intensive to super-intensive) which was encouraged by the increased market demand and price, and availability of good feeds.

Unfortunately, this industry has suffered drastic collapses from decreased growth and survival in over-

crowded shrimp cultures. Studies indicated that 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).

Shrimp immunity is another important concern in shrimp culture. There is no specific immunity (immunoglobulin) in shrimp, which is using non-specific immunity to resistant disease and virus. Non-specific immunity factors, such as phenoloxidase (PO), superoxide dismutase (SOD), peroxidase (POD), antibacterial activity (Ua), lysozyme (Ul), play an important function at the immune response and disease resistance of shrimp (Wang and Wu, 2000; He, 2004).

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 and Browdy, 2001). Studies indicate that artificial substrates could increase the natural food supplement for shrimp, improve the water quality of ponds, and control the disease bacteria as biofilms (Thompson *et al*., 1999, 2002; Bratvold and Browdy, 2001; Burford *et al*., 2004; Preto *et al*., 2005; Arnold *et al*., 2005, 2006; Zarain-Herzberg *et al*., 2006; Ballester *et al*., 2007; Arnold *et al*., 2009). No research about the effects of artificial substrates on shrimp non-specific immunity factors was reported.

Therefore, the primary aim of the present work was to investigate the effects of artificial substrates on the shrimp growth, survival, spatial distribution and non-specific immunity factors in intensive *Litopenaeus vannamei* culture.

## Materials and Methods

### Origin of Shrimp and Study Site

The experiment was carried out from 12 June to 12 August, 2008 in Hai-yue Shrimp Company of Yangjiang, China. The shrimp used in this study were obtained from the same spawner in a local commercial hatchery. Prior to the experiment, the shrimp were transferred into PVC aquaria (1.0×1.0×1.5 m, water volume 1000 L) and underwent a 7-day acclimation period. The shrimp (30-day old *Litopenaeus vannamei* post-larvae) with average (±SD) weight was 0.015 ± 0.003 g at the beginning of study.

### Experimental Design

Eight PVC aquaria (1.0×1.0×1.5 m, water surface area 1 $m^{-2}$, water volume 1000 L) were used to test four treatments ( in two replicates): Group 0 (G0), as a control group without artificial substrates, and Group 1 (G1), Group 2 (G2) and Group 3 (G3) with 1, 2 and 3 pieces of artificial substrates respectively. The shrimp stocking density was 500 shrimp $m^{-2}$ of water surface area (500 shrimps per aquarium).

The structure of artificial substrates was based on the description of previous study (Ballester *et al*., 2007) (Figure 1). It consisted of two modified polypropylene fabrics screens (50 × 100 cm, 0.2 cm thickness) with a large rough surface area and loose porous inner structure. The substrates were fixed to a PVC 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 20 cm distance between them (G2 and G3).

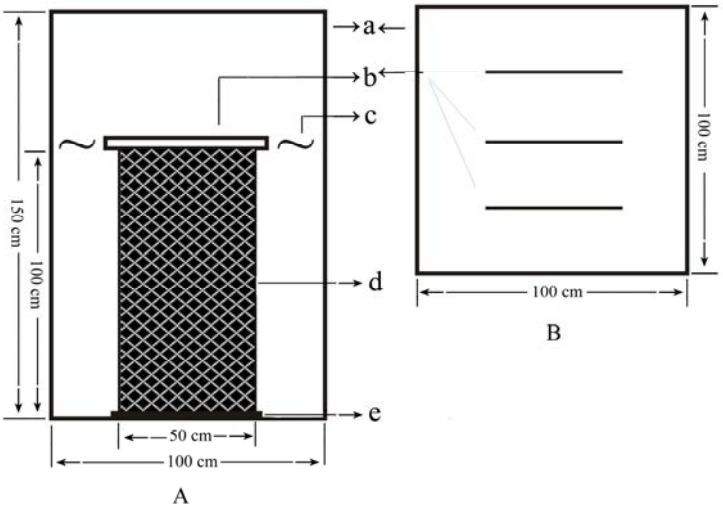


**Figure 1.** The side view (A) of artificial substrates in aquarium, and the vertical view (B) of artificial substrates in Group 3 (G3) with 3 artificial substrates. a- aquarium, b- PVC pole, c-water surface, d-artificial substrates, e-plumb ballasts.

**Experimental Preparation and Rearing Management**

Water changes were made four times daily at 0:00, 8:00, 14:00 and 20:00 to all the treatments, and 10% of the water (by volume) was renewed every time to maintain water quality. The seawater was provided from the same water source after sand filtration. During the course of the experiment, the culture water was filtered except during feeding time. Additionally, each aquarium was supplied with an underwater continuous gentle aeration.

Commercial shrimp feed (Haima$^{TM}$, 40% protein, 8% lipid) was provided four times daily at 6:00, 12:00, 18:00 and 22:00. The amount of feed was adjusted daily for each aquarium based upon the amount of uneaten food observed.

**Experimental Procedure and Sample Collection**

Water quality parameters were measured twice a day (06:00 and 18:00). The following parameters were tested: pH with a portable pH meter (Hanna HI 991003), water temperature (WT) 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 ($NH_3$-N) and nitrite ($NO_2$-N) were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA 1993).

To observe spatial distribution of shrimp in water, all treatments were taken pictures twice daily (10:00 and 16:00) by a digital camera (Canon IXUS 70). The pictures were downloaded to a computer with Canon View software provided with the camera. The total number of shrimp and the quantity of shrimp attached on artificial substrates were calculated according to pictures. Then the percentage of shrimp attained on artificial substrates was analyzed. In order to analyze easily, the mean percentage of every 10-day was compared among treatments.

At the end of the 60 day experiments, shrimp average weights and survival rates were measured. Meanwhile, 50 shrimp were randomly sampled from each aquarium for non-specific immunity factors measure. Haemolymph was withdrawn from the ventral sinus of shrimp into 1 ml sterile syringe. Haemolymph from same aquarium (50 shrimp) was pooled, which was centrifuged at 5000 rpm at 4°C for 10 min after 10 h placed at 4°C. The supernatant fluid, namely serum, was distracted into 1.0 mL Eppendorf tube, which was used for enzyme activity tests. Non-specific immunity factors of haemolymph serum, including phenoloxidase (PO), superoxide dismutase (SOD), antibacterial activity (Ua), lysozyme (Ul), hemolysin and peroxidase (POD) were measured according to the previous methods (Li *et al*., 2006).

**PO:** 10 µl blood serum, 200 µl phosphate buffer (0.013 M $K_2HPO_4$, 0.087 M $KH_2PO_4$, pH 6.0) and 10 µl L-DOPA solution (Sigma) were added into 96-microtitor plate in turn. Shaken 4 times in Microplate Spectrophotometer (550, Bio-Rad), absorbance value ($OD_{490}$) was read at wavelength of 490 nm every 4 min. $OD_{490}$ increase 0.001 $min^{-1}$ is regarded as one activity unit.

**SOD:** Added 10 µl 50 mM pyrogallol autoxidation to 4.5 ml phosphate butter (0.048 M $K_2HPO_4$, 0.02 M $KH_2PO_4$, pH 8.3), shook up quickly, then absorbance value ($OD_{325}$) was read at wavelength of 325 nm every 30 s, autoxidation rate was modulated to about 0.070 OD $min^{-1}$. SOD activity was measured as follows: added 50 µl serum to 4.5 ml phosphate buffer before adding pyrogallol autocidation, shook up quickly, then read $OD_{325}$ every 30 s. One SOD activity unit was defined as the amount of enzyme, which inhibited the pyrogallol autoxidation rate by 50% in 1 ml reaction solution per min.

POD: 20 µl serum and 180 µl color-developing buffer (7.3 g $C_6H_8O_7 \cdot H_2O$, 11.86 g $Na_2HPO_4 \cdot 2H_2O$, 1000 ml $H_2O_2$) were added into 96-well microtiter plate in turn. $OD_{490}$ ($A_3$) was read at wavelength of 490 nm. Took out 96-well microtiter plate and added 20 µl color-developing reagent (4 mg $C_6H_8N_2$, 4 µl 30% $H_2O_2$, 10 µl color-developing buffer) into the mixture, shook up 12 times in Microplate Spectrophotometer, color-developing 15 min in dark, then $OD_{490}$ ($A_4$) was read. Relative POD activity was expressed as $A_4 - A_3$.

**Ua:** *Escherichia coli* D31 was culture in minimal medium at 26°C 2 days before assay. As substrate, *E.coli* D31 was rinsed from medium by phosphate buffer (0.028 M $K_2HPO_4$, 0.072 M $KH_2PO_4$, pH 6.4), and preparing suspension ($OD_{570}$=0.3-0.5). 3 ml suspension was transferred into test tube in an ice-water bath, then 50 µl serum was added, mix uniformity, and absorbance value ($A_0$) was read at wavelength of 570 nm quickly. The test tube was placed in a water bath at 37°C for 30 min., and then transferred to an ice-water bath to stop reaction; read absorbance value ($A$) at wavelength of 570 nm. Ua was calculated following the formula, $Ua=[(A0 - A)/A]^{1/2}$.

**Ul:** *Micrococcus lysodeikticus* (Sigma) was added to phosphate buffer (0.028 M $K_2HPO_4$, 0.072 M $KH_2PO_4$, pH 6.4) and modulated $OD_{570}$ of the suspension to 0.3. 3 ml suspension was transferred into a test tube in an ice-water bath, then 50 µl serum was added, mixed uniformity, and absorbance value ($A_0$) was read at wavelength of 570 nm quickly. The test tube was placed in a water bath at 37°C for 30 min., and then transferred to an ice-water bath to stop reaction; read absorbance value ($A$) at wavelength of 570 nm. Ul was calculated following the formula, $Ul=(A_0 - A)/A$.

**Hemolysin:** Fresh chicken blood was collected, and Alsever's solution (2.05 g glucose, 0.8 g $Na_3C_6H_5O_7 \cdot 2H_2O$, 0.42 g NaCl, make up to 100 ml with distilled water) was used to hold back blood

coagulation. The diluted blood corpuscle was centrifuged at 2000 rpm for 5 min, the supernatant fluid was discarded and red blood cell was rinsed to preparing suspension using 3% normal saline. 50 µl shrimp serum mixed with 450 µl 3% normal saline, then mixed with 2 ml suspension. 500 µl 3% normal saline mixed with 2 ml suspension as blank control solution. The mixture and blank control solution were placed in a water bath at 25°C for 1 h, and shaken up several times during the process, and then placed into an ice-water bath to stop reaction; read absorbance value ($OD_{540}$) at wavelength of 540 nm. $OD_{540}$ increase 0.001 is regarded as one activity unit.

### Data Analysis

Water quality data, the distribution percentage of shrimp attached on artificial substrates, shrimp weight, survival and yield 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. Non-specific immunity factors data figured using Origin 7.5 and analyzed by one-way ANOVA using SPSS 16.0.

## Results

### Water Quality Parameters

Water quality parameters fluctuated during the experiment period (Table 1). None of the monitored water quality parameters were significantly ($P<0.05$) affected by artificial substrates during the study. In all the treatments each water quality parameter changed only slightly during the experimental period and was within the suitable range recommended for culturing penaeid shrimp.

### Growth Parameters

The shrimp average weight, survival and yield of G1, G2 and G3 were significantly ($P<0.05$) higher than those of G0, and those parameters increased significantly ($P<0.05$) as the number of artificial substrates increased (Table 2).

### The Percentage of Shrimp Attained on Artificial Substrates

The percentage of shrimp attained on artificial substrates was showed in Figure 2. During the course of experiment, the percentage of shrimp on artificial substrates increased with an increasing number of artificial substrates. There were no significant differences ($P<0.05$) between the percentages of shrimp on artificial substrates for first 10-day. During second and third 10-day, the percentages of G2 and G3 were higher significantly ($P<0.05$) than G1. During $4^{th}$, $5^{th}$ and $6^{th}$ 10-day, there were significant difference ($P<0.05$) among treatments.

### Non-Specific Immunity Factors

### Peroxidase Activity

Peroxidase (POD) activities of G1, G2 and G3 were lower significantly ($P<0.05$) than that of G0. The POD activity decreased with an increasing number of artificial substrates, but no significant effect ($P<0.05$) was observed between G1, G2 and G3 (Figure 3).

### Lysozyme Activity

Lysozyme activities of G1, G2 and G3 were lower significantly ($P<0.05$) than that of G0. Lysozyme activity decreased with an increasing

**Table 1.** Condition of water quality parameters during the course of the experiment

| Treatments | WT (°C) | 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 |
| G2 | 24.7-26.7 | 26.0-27.3 | 74.-7.7 | 7.5-8.6 | 0.15-0.21 | 0.03-0.10 |
| G3 | 26.6-27.3 | 24.6-25.7 | 7.2-7.6 | 7.6-8.5 | 0.18-0.23 | 0.04-0.09 |

**Table 2.** Effects of artificial substrates on growth, survival and yield in *Litopenaeus vannamei*

| Treatments | Weight gain (g shrimp $^{-1}$) | Survival (%) | Yield (g $m^{-2}$) |
|---|---|---|---|
| G0 | $3.35\pm0.18^{a}$ | $63.50\pm0.02^{a}$ | $1044.37\pm19.76^{a}$ |
| G1 | $3.73\pm0.16^{b}$ | $70.50\pm0.02^{b}$ | $1313.93\pm96.84^{b}$ |
| G2 | $4.41\pm0.11^{c}$ | $76.00\pm0.01^{c}$ | $1674.28\pm71.45^{c}$ |
| G3 | $5.45\pm0.06^{d}$ | $86.50\pm0.02^{d}$ | $2370.40\pm82.59^{d}$ |

G0, G1, G2 and G3 denote the treatment with 0, 1, 2 and 3 pieces of artificial substrates, respectively. Different superscripts denote significant difference ($P<0.05$) between treatments.

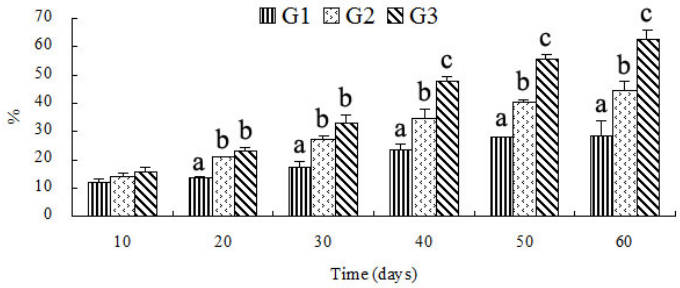


**Figure 2.** Mean percentage of every 10-day of *Litopenaeus vannamei* attained on artificial substrates for 60 days in treatments with 1 (G1), 2 (G2) and 3 (G3) artificial substrates. Different superscripts denote significant difference (P<0.05) within cultivating days.

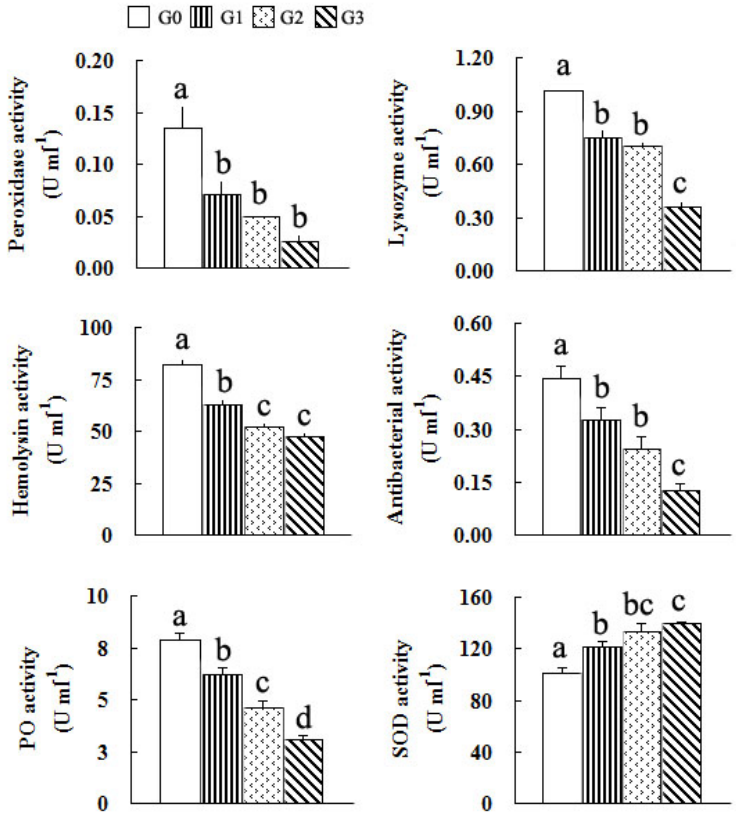


**Figure 3.** Effects of artificial substrates on phenoloxidase (PO), superoxide dismutase (SOD), antibacterial activity, lysozyme activity, hemolysin activity and peroxodase (POD), in the serum of *Litopenaeus vannamei*. G0, G1, G2 and G3 denote the treatment with 0, 1, 2 and 3 pieces of artificial substrates, respectively. Different superscripts denote significant difference (P<0.05) between treatments.

number of artificial substrates, and Lysozyme activity of G3 was significantly lower (P<0.05) than those of G1 and G2, but no significant effect (P<0.05) was observed between G1 and G2 (Figure 3).

**Hemolysin Activity**

Hemolysin activities of G1, G2 and G3 were lower significantly (P<0.05) than that of G0. Hemolysin activity decreased with an increasing number of artificial substrates, and hemolysin activity of G1 was significantly higher (P<0.05) than those of G2 and G3, but no significant effect (P<0.05) was observed between G2 and G3 (Figure 3).

**Antibacterial Activity**

Antibacterial activity (Ua) activities of G1, G2 and G3 were lower significantly (P<0.05) than that of G0. Ua decreased with an increasing number of artificial substrates, and Ua of G3 was significantly lower (P<0.05) than those of G1 and G2, but no significant effect (P<0.05) was observed between G1and G2 (Figure 3).

**Phenoloxidase Activity**

Phenoloxidase (PO) activity of G1, G2 and G3 was higher significantly ($P<0.05$) than that of G0. Moreover, PO activity decreased significantly ($P<0.05$) as artificial substrates increased (Figure 3).

**Superoxide Dismutase Activity**

Superoxide dismutase (SOD) activity of G1, G2 and G3 was lower significantly ($P<0.05$) than that of G0. SOD activity decreased significantly ($P<0.05$) with an increasing number of artificial substrates (Figure 3).

## Discussion

Some studies indicated that artificial substrates could improve the water quality in the shrimp culture system. However, none of the monitored water quality parameters were significantly ($P<0.05$) affected by artificial substrates in the present study. Moreover, all monitored water quality parameters were with "safe" level recommended for optimal growth and survival of penaeid shrimp (Chen and Lei, 1990; Chien, 1992). It maybe benefited from the good management of water quality. Continuous filtering could quickly remove the undesirable sediment. Meanwhile, multiple-low-dose water exchange decreased the concentration of the harmful substances and maintained the stable water quality parameters. Moreover, a continuous gentle aeration provided plenty of dissolved oxygen to avoid toxic matter.

Many studies have illustrated that artificial substrates could increase shrimp growth and survival (Sandifer *et al*., 1987; Tidwell *et al*., 1998; Peterson and Griffith, 1999; Bratvold *et al*., 2001; Moss and Moss, 2004; Arnold *et al*., 2006; Zarain-Herzberg *et al*., 2006; Ballester *et al*., 2007; Arnold *et al*., 2009). The conclusion was strengthened by the results of this study. In the present study, shrimp weight gain, survival rate and yield were affected significantly ($p<0.05$) by the artificial substrates. Moreover, those growth parameters increased significantly ($P<0.05$) with the number of artificial substrates increased.

Being benthic animals, shrimp are constrained to two-dimensional space rather than three-dimensional volume (Kumlu *et al*., 2001). Authors of some studies have speculated that the artificial substrates could lessen the negative effect by enlarging the living space for the shrimp, but direct evidence was lacking (Tidwell *et al*., 1998, 1999; Kumlu *et al*., 2001; Arnold *et al*., 2005, 2006; Zarain-Herzberg *et al*., 2006). The conjecture was proven directly by the result of this study. In present study, the polypropylene fabrics screen fixed vertically in the water column was used as artificial substrates, and the water transparency was high. So during non-feeding period, the percentage of shrimp attached on artificial substrates could count directly from pictures. With an increasing number of artificial substrates, more shrimp attached on artificial substrates. Moreover the trends were more significant as rearing days increased. It was likely that artificial substrates could meet the demand of shrimp which need more living space and natural food with culturing time.

As important health parameters, non-specific immunity factors were affected by different environmental conditions, such as DO, pH, sulfide, salinity, nitrite, ammonia and water temperature (Cheng and Chen, 2000; Liu and Chen, 2004; Tseng and Chen, 2004; Cheng *et al*., 2005; Jiang *et al*., 2005; Wang and Chen, 2005, 2006; Cheng *et al*., 2007; Hsu and Chen, 2007; Li and Chen, 2008). Study demonstrated that both PO activity and hemolysin activity were affected by stocking density, and PO activity was increased and hemolysin activity was decreased with increasing stocking density (Li *et al*., 2006). In the present study, artificial substrates could increase the SOD activity, but reduce phenoloxidase (PO), antibacterial activity, lysozyme activity, hemolysin activity and peroxodase (POD). Moreover, differences of those non-specific immunity factors changed significantly ($P<0.05$) with an increasing number of artificial substrates. It was suggested that artificial substrates could affect those non-specific immunity factors by alleviating the negative effect of the high stocking density.

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## References

APHA (American Public Health Association) 1993. Standard Methods for the Examination of Water and Wastewaters, 17th edition. American Public Health Association, Washington, DC.

Arnold, S.J., Coman, F.E., Jackson, C.J. and Groves, S.A. 2009. High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. Aquaculture, 293: 42-48.

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.

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.

Cheng, S.Y., Hsu, S.W. and Chen, J.C. 2007. Effect of sulfide on the immune response and susceptibility to *Vibrio alginolyticus* in the kuruma shrimp *Marsupenaeus japonicus*. Fish and Shellfish Immunology, 22: 16-26.

Cheng, W. and Chen, J.C. 2000. Effects of pH, temperature and salinity on immune parameters of the freshwater prawn *Macrobrachium rosenbergii*. Fish and Shellfish Immunology, 10: 387-391.

Cheng, W., Wang, L.U. and Chen, J.C. 2005. Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to *Vibrio alginolyticus*. Aquaculture, 250: 592-601.

Chien, Y.H. 1992. Water quality requirements and management for marine shrimp culture. In: J Wyban (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.

He, N.H. 2004. The establishment and application of immune indexes of penaeid shrimp. Journal of Xiamen University, 43: 385-388 (in China, with English abstract).

Hsu, S.W. and Chen, J.C. 2007. The immune response of white shrimp *Penaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under sulfide stress. Aquaculture, 271: 61-69.

Jiang, L.X., Pan, L.Q. and Bo, F. 2005. Effect of dissolved oxygen on immune parameters of the white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology, 18: 185-188.

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.

Li, C.C. and Chen, J.C. 2008. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under low and high pH stress. Fish and Shellfish Immunology, 25: 701-709.

Li, Y.Q., Li, J. and Wang, Q.Y. 2006. The effects of dissolved oxygen concentration and stocking density on growth and non-specific immunity factors in Chinese shrimp, *Fenneropenaeus chinensis*. Aquaculture, 256: 608-616.

Liu, C.H. and Chen, J.C. 2004. Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. Fish and Shellfish Immunology, 16: 321-334.

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*. J. 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.

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

Tseng, I.T. and Chen, J.C. 2004. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under nitrite stress. Fish and Shellfish Immunology, 17: 325-333.

Wang, F.I. and Chen, J.C. 2006. Effect of salinity on the immune response of tiger shrimp *Penaeus monodon* and its susceptibility to *Photobacterium damselae* subsp. *damselae*. Fish and Shellfish Immunology, 20: 671-681.

Wang, J.P. and Wu, X.F. 2000. Status of study on the haemocyte and humoral immunity of shrimp (in China). J. of Zhejiang Ocean University, 19: 354-360.

Wang, L.U. and Chen, J.C. 2005. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. Fish and Shellfish Immunology, 18: 269-278.

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.