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Influence of the Artificial Substrates on the Attachment Behavior of *Litopenaeus vannamei* in the Intensive Culture Condition

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Abstract: This study evaluated the influence of artificial substrates on the attachment behavior of *Litopenaeus vannamei* reared in the intensive culture condition. *L. vannamei* were grown from PL60 (60-day-old postlarvae) for 8 weeks at high density (500 shrimp per m^2) in 12 independent aquaria (1.0×1.0×1.5 m, water surface area 1 m^{-2}, water volume 1000 l). The experimental design consisted of four treatments: Group A (GA), artificial substrates were immersed in water all the time; Group B (GB), artificial substrates immersed in water were taken out of water weekly and returned immediately; Group C (GC), artificial substrates were exchanged weekly by new one, and Group D (GD) without artificial substrates. With a longer rearing time, the increase of the percentage of shrimp attachment on artificial substrates demonstrated continuous in GA but discontinuous in GB and GC. Meanwhile, based on the mean of weeks, the percentage of shrimp attachment on artificial substrates in GA was significantly higher than those in GB and BC from the second week. The final weight, survival rate and final biomass of the shrimp reared in the treatment with artificial substrates were significantly higher than those in other treatment without artificial substrates. However, there was not significant difference in Food Conversion Rate (FCR) among different experimental treatments. So, we suggested that the differences of shrimp growth parameters were affected mainly by the living space added with the addition of artificial substrates.

Key words: Aquaculture, growth, high density, living space, survival, vertical surface, white shrimp

INTRODUCTION

Because of the high demand for shrimps in Japan, the United States and Europe, shrimp aquaculture has expanded rapidly in all around the world, especially in tropical areas, such as Southeast Asia and Latin America (Paquotte *et al.*, 1998; Lombardi *et al.*, 2006). Among all species of shrimp, *L. vannamei*, which represent over 90% of shrimp culture in the Western hemisphere, is the most commonly cultured shrimp in Central and South American countries, China, and Thailand (Frias-Espericueta *et al.*, 2001; McGraw *et al.*, 2002; Saoud *et al.*, 2003; Wurmann *et al.*, 2004; Cheng *et al.*, 2006). Unfortunately, this industry has suffered drastic collapses from decreased growth and survival as an increase in stocking density. Reduced growth and survival of shrimp cultured at high densities is thought to result 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).

During the last 10 years, an innovative technology, by adding artificial substrates to penaeid shrimp culture systems in attempt to overcome the negative effect of increased stocking density on growth and survival, has been undertaken in USA, Turkey, Australia, Brazil and China (Bratvold and Browdy, 2001; Kumlu *et al.*, 2001; Moss and Moss, 2004; Arnold *et al.*, 2005, 2006; Ballester *et al.*, 2007; Zhang *et al.*, 2010). For the advantage of the artificial substrates on shrimp growth, different authors who carried out experiments from various aspects obtained different results and gave different explanation including improvement of the water quality, addition of the natural food supplement, limitation of pathogenic bacteria reproduce, provide refuge for shrimp to escape any negative behavioural interactions and adding living space (Tidwell *et al.*, 1998, 1999; Thompson *et al.*, 1999, 2002; Bratvold and Browdy, 2001; Kumlu *et al.*, 2001; Ballester *et al.*, 2007; Burford *et al.*, 2004; Preto *et al.*, 2005; Arnold *et al.*, 2005, 2006; Zarain-Herzberg *et al.*, 2006). Up to now, however, there is not an agreement about the predominant factor among those factors. For example, the result by Ballester *et al.* (2003) determined that growth and survival of shrimp were not enhanced in the presence of floated cages that had their biofilm periodically removed, suggested that the importance of using substrates for shrimp is not related to the space but to the availability of food provided by biofilm formed on the substrate, while recent study by Zhang *et al.* (2010), which studied the effects of artificial substrates on the spatial distribution of shrimp in the intensive culture condition, suggested that the difference of the shrimp growth and survival were affected mainly by living space added with the addition of artificial substrates. Therefore, a better understanding of the effect of artificial substrates on shrimp performance is necessary.

In light of this, the present study aimed to evaluate the advantage of artificial substrates for overcoming the negative effect of increased stocking density on growth parameters by analyzing the shrimp attachment behavior with the addition of artificial substrates.

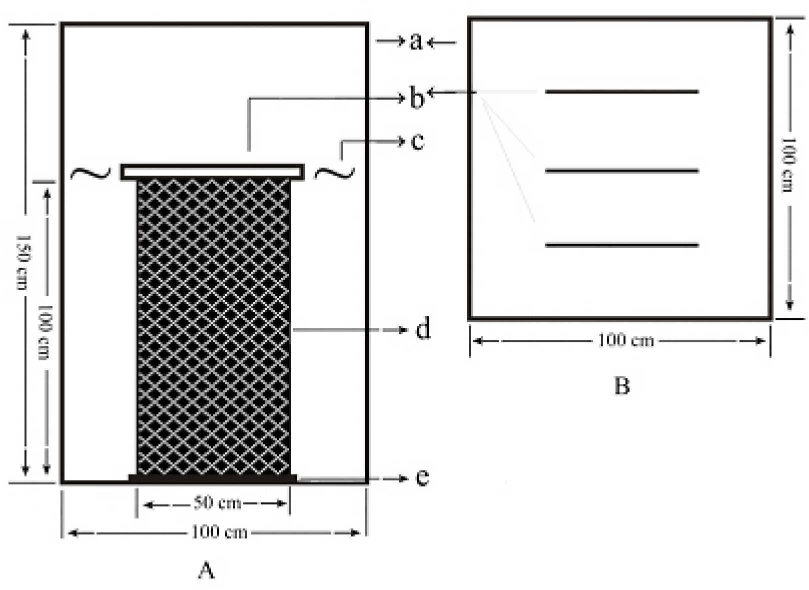
MATERIALS AND METHODS

Origin of shrimp and study site: The experiment was carried out for 8 weeks from July 05th, to August 29th, 2010 in Hai-yue Shrimp Cultivate Company of Yangjiang, China. The shrimp used in this study were obtained from the same spawner bredeer 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 (60-day old *L. vannamei*) average (±SD) weight is 1.21 ± 0.06 g at the beginning of study. The shrimp stocking density were 500 shrimp m^{-2} of water surface area (500 shrimp/each aquarium).

Experimental design: Twelve 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 (3 replicate): Group A (GA), artificial substrates were immersed in the culture water all the time; Group B (GB), artificial substrates immersed in water were taken out of water weekly and returned immediately; Group C (GC), artificial substrates were exchanged weekly by new one, and Group D (GD) without artificial substrates.

The structure of artificial substrates was based on the description of previous study (Zhang *et al*., 2010) (Fig. 1). It consisted of three groups of vertical surface, and each vertical surface contained 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.

Experimental preparation and rearing management: To keep the same water quality condition for all trial shrimp, all aquaria were connected by pipes and culture water was flow free among them, but shrimp was limited respectively. Water changes were made four times daily at 0:00, 8:00, 14:00 and 20:00, 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.



a: aquarium; b: PVC pole; c: water surface; d: artificial substrates; e: plumb ballasts

Fig. 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

One feeding tray was set up in each aquarium. Shrimps were fed the same commercial pellets (Yuehai^{TM}) containing crude protein (40%), crude fat (4%), crude fiber (5%), moisture (12%) and ash (16%). Feed was delivered exclusively on the feeding tray in order to be able to estimate consumption. Visual estimates of feed consumption were made 2 h after each meal and by the same person to avoid any skew. The feed was provided four times daily at 6:00, 12:00, 18:00 and 22:00, and the feeding rate 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).

The methods to observe the shrimp attachment behavior was based on the description of previous study (Zhang *et al.*, 2010). 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 in the aquarium and the quantity of shrimp attached on artificial substrates were calculated according to pictures. Then the percentage of shrimp attained on artificial substrates and the shrimp survival rates were analyzed. The final mean weight of shrimp was obtained by measuring all shrimp wet weight with sensitive balance at the end of the experiment. The percentage of shrimp attained on artificial substrates, growth, survival rates, final biomass, and feed conversion ratio were calculated as follows:

Attachment Percentage (AP%) =
(the quantity of shrimp attached on artificial substrates/ the total number of shrimp in the aquarium) ×100

Growth rate (GR) =
(final mean weight - initial mean weight)/time (days)

Survival (S%) =
(final number of shrimp/initial number of shrimp)×100

Final biomass =
final number of shrimp × final mean weight

Feed Conversion Ratio (FCR) = feed provided /weight gain

Statistical analysis: Water quality data, the percentage of shrimp attained on artificial substrates, final weight, survival, final biomass, and feed conversion ratio 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

Water quality parameters: Water quality parameters fluctuated slightly during the experiment period (Table 1). None of the monitored water quality parameters changed significantly during the study, and each water quality parameter was within the suitable range recommended for culturing penaeid shrimp.

Growth parameters: After 8 weeks of grow out culture, the artificial substrates had a significant influence on the growth of the shrimp (Table 2). At harvest, the final weight, survival rate and final biomass of the shrimp reared in GD without artificial substrates were significantly lower than those in other treatments with artificial substrates. Meanwhile, the final weight and final biomass from the GA were significantly higher than GB and GC, but there was no significant difference between GB and GC. Food Conversion Rate (FCR) was not significant difference among different experimental condition (Table 2).

The percentage of shrimp attained on artificial substrates: The percentage of shrimp attained on

Table 1: Condition of water quality parameters during the course of the experiment

Treatments	WT (ºC)	Salinity (‰)	pH	DO (mg/L)	NH_3-N (mg/L)	NO_2-N (mg/L)
GA	24.6-26.1	25.2-26.8	7.3-7.6	7.9-8.3	0.15-0.20	0.08-0.12
GB	25.2-27.3	24.6-25.2	7.4-7.8	7.7-8.0	0.16-0.21	0.07-0.10
GC	25.7-26.8	26.5-27.7	7.5-7.7	7.8-8.2	0.17-0.22	0.04-0.09
GD	24.1-26.3	25.5-26.3	7.5-7.8	7.5-7.9	0.16-0.22	0.05-0.08

Table 2: Mean values (± SD) of zootechnical parameters of *Litopenaeus vannamei* for treatments

Treatments	Final weight (g)	Survival (%)	Final biomass (g/m^2)	FCR
GA	13.27±1.16^a	87.86±1.02^a	5833.12±582.80^a	0.88±0.37
GB	10.53±0.69^b	88.45±2.11^a	4652.32±263.76^b	0.91±0.24
GC	9.07±0.28^b	85.63±2.74^a	3887.79±289.40^b	0.90±0.32
GD	6.63±1.14^c	59.03±3.56^b	1972.15±479.46^c	0.88±0.46

Different superscripts denote significant difference (p<0.05) among treatments

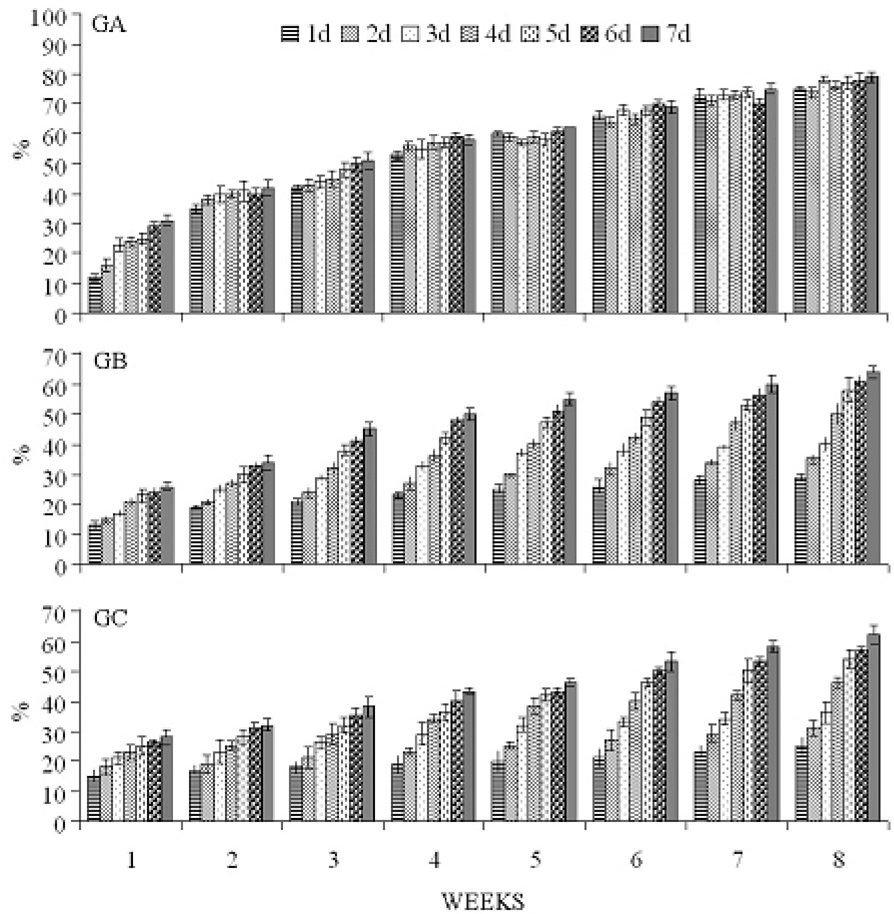


Fig. 2: Mean percentage of every day of *Litopenaeus vannamei* attained on artificial substrates for 8 weeks in group A (GA), group B (GB) and group C (GC)

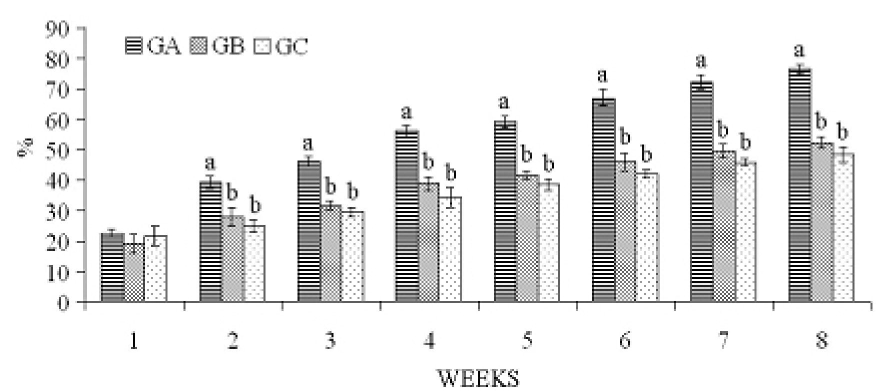


Fig. 3: Mean percentage of every week of *Litopenaeus vannamei* attained on artificial substrates for 8 weeks in group A (GA), group B (GB) and group C (GC). Different superscripts denote significant difference ($p<0.05$) within cultivating days

artificial substrates was showed in Fig. 2. During the course of experiment, the percentage of shrimp on artificial substrates in all treatments increased with a longer rearing time, which appeared continuous in GA but discontinuous in GB and GC. Based on the mean of weeks, the percentage of shrimp on artificial substrates in

GA was significant higher than those in GB and BC from the second week to the end, but there was not significant different between GB and GC (Fig. 3).

DISCUSSION

As one of key factors for culture shrimp, water quality not only affects the shrimp growth and survival rate, but also affects the accuracy of the experiment result (Chim *et al*., 2008). In this study, several measures were taken to increase the water quality. Firstly, all aquaria were connected by pipes and water was flow free among them. It was sure that all trial shrimp were provided for the same water quality condition and decrease the zootechnical biases. Secondly, continuous filtering could quickly remove the undesirable sediment from the culture system and maintained a slight flow in aquarium which was the similar to the natural environment for shrimp. Thirdly, multiple-low-dose water exchange decreased the concentration of the harmful substances and maintained the stable water quality parameters. Lastly but not least, a continuous gentle aeration provided plenty of dissolved oxygen to avoid toxic matter producing. Therefore, in the study, the known limiting factors of water quality such as dissolved oxygen, ammonia, nitrite, water temperature, pH and salinity were within “safe” level recommended for optimal growth and survival of penaeid shrimp (Chen and Lei, 1990; Chien, 1992).

Direct observe with the naked eye and manual note are important methods to study the shrimp behavior with a long time. However, this method is highly invasive, and requires the animal to be immobilized and partially damaged (Kruk, 1997). To minimize the manual error of experiment result, digital camera was used to investigate the shrimp attachment behavior in the study. The camera, which was fixed to a steel bar above tanks during the days of culture, could not affect the shrimp natural live. Meanwhile, good management of water quality which maintained the culture water high transparence kept the shrimp attachment behavior to be observed directly by camera. Thus, both the total number of living shrimp in the aquarium and the amount of shrimp attained on the artificial substrates could be calculated from the photo. On the other hand, the time taken picture was selected at 10:00 and 16:00 daily, which was not the trial shrimp feeding time but relaxing time. So the result could really indicate the shrimp attachment behavior.

A lot of studies used different artificial substrate types such as fiberglass window screen, plastic mesh and commercial artificial substrates under a variety of shrimp culture conditions indicated that artificial substrates could improve the growth and survival rate of the shrimp (Sandifer *et al*., 1987; Tidwell *et al*., 1998; Peterson and Griffith, 1999; Bratvold and Browdy, 2001; Moss and Moss, 2004; Arnold *et al*., 2006; Zarain-Herzberg *et al*., 2006; Ballester *et al*., 2007). The conclusion was strengthened by the result of this study. The present results demonstrated that higher survival and higher weight as well as higher biomass were obtained in the grow-out rearing of *L. vannamei* in a high density culture system where vertical surface of polypropylene fabrics were provided.

Some studies reported that artificial substrates could decrease the shrimp Feed Conversion Ratio (FCR) by forming biofilm to increase the natural food supplement for shrimp (Bratvold and Browdy, 2001; Ballester *et al*., 2003, 2007). However, the FCR obtained in our trials could not be affected significantly by the addition of the artificial substrates in the shrimp culture system. Meanwhile, the biofilm on artificial substrates removed weekly also could not affect the FCR significantly in present study. It can be attributed to the following measures. On one hand, the shrimp in present trial were provided enough commercial artificial feed with sufficient nutrition by feeding four times daily, while others just were not applied for more than two times (Bratvold and Browdy, 2001; Ballester *et al*., 2003, 2007). On the other hand, Feed was delivered exclusively on the feeding tray, and the feed rations of next meal were adjusted by visual estimates of feed consumption. This accurate and frequent alteration in feed ration led to a reduction of waste (Smith *et al*., 2002; Chim *et al*., 2008).

In the present study, we test the predominant factor by analysis the change of shrimp attachment percentage under the condition of frequent exchange of the artificial substrates. Results indicated that frequent exchange of the artificial substrates in the culture system would affect the shrimp attachment significantly. Once the shrimp were forced to leave the artificial substrates, it may be take the shrimp time to make a new attachment to the artificial substrate even the biofilm being on the artificial substrates could not be destroyed. During the course of the attachment, a large number of shrimp could be assembled on the aquaria bottom from the artificial substrates (Zhang *et al*., 2010). As a result, it would be appeared some of the negative effects of increased stocking density, which included a decrease of favorable space and natural food sources, and an increase in adverse shrimp behavior such as cannibalism. The conclusion was supported by the lower survival rate and lower growth in those aquaria where the artificial substrates was exchanged weekly. Therefore, the present result suggested that the shrimp were benefited from the present of living space added with the addition of artificial substrates.

Above of all, because the shrimps in all aquaria were supplied with suitable water quality and adequate nutritional food, we suggested that the differences of shrimp growth parameters were affected mainly by the living space added with the addition of artificial substrates.

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