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Comparisons among double-, triple-, and hexa-layer net cages on the cultivation of white leg shrimp *Litopenaeus vannamei* in an indoor zero water exchange system

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abstract

This study was conducted to compare the survival and growth of white leg shrimp, *Litopenaeus vanname*, cultured in three types of indoor multi-layer net cages, namely double-, triple-, and hexa- net cages of the same volume. Total rearing duration was 57 days, with an initial density of 64/m^2 for each layer. Salinity and water temperature were maintained at 25 ± 1 ppt and 29.1-31.6°C, respectively. The initial body weight of the shrimp was 0.15 g. Results indicated that the hexa-layer net cage was the best for spatial usage of the entire volume of water, with a total production 1.33 and 2.44 times that of the triple- and double-layer ones, separately. The average survival rates of each layer in the hexa- (75.9%), triple- (73.4%), and double-layer (80.5%) net cages did not significantly differ ($p > 0.05$). There were no differences in harvest size among the three treatments, either. However, the proportion of large-sized shrimp was highest in the hexa-layer cage among the three types of cages. The feed conversion ratio was less than 0.5, and the reasons for this notable result are also discussed.

Key words: Feed conversion ratio, Growth rate, Multi-layer net cage, Survival rate, Unit production

Introduction

So far, only a few tests have been carried out on shrimp cage culture (Paquotte *et al.*, 1998). Most of them were devoted to net rearing, particularly in floating and the number of tests was too limited to extrapolate the results to economically feasibile applications. Marques *et al.* (2000) studied the optimum stocking density for the nursery phase culture of the freshwater prawn *Macrobrachium roserbergii* in cages within an earthen pond. Their results seemed to identical that it would be feasible to reduce the costs normally encountered in conventional nursery systems. They also mentioned that nursery and grow-out cultures of *M. rosenbergii* had already been successfully tested in cages in India as early as 1981.

For penaeids, Shanmugam *et al.* (1994) used 5×4×1 m net cages to culture *Penaeus monodon* in India at a density of

12 individuals/m^2 for 69 days. The shrimp grew from 1.5 to 17.8 g on average. But 10 days before harvest, all of shrimp were lost in a large storm. A cage culture of *P. indicus* for 90 days was achieved by Shanmugam *et al.* They produced a total harvest 3.7 kg/20 m^2/90 days with 100% survival (Shanmugam *et al.,* 1995). In order to design a new technology for culturing shrimp in floating cages in an estuarine zone, Paquotte *et al.* (1998) carried out an intensive culture of *Litopenaeus vannamei,* which had been reared in different densities in cages and during the wet and dry seasons in an estuarine zone in the state of Bahia, Brazil. The average growth rate was about 0.8 g/wk, and the average final biomass was 800 g/m^2.

As early as 1974, Forster and Beard carried out experiments to assess the suitability of prawns for indoor intensive cultivation (Forster *et al.*, 1974); their experiments were run in closed systems comprising tanks, a reservoir of glass fiber, and a biological filter. Water was circulated from the reservoir and percolated through the filter before passing through the experimental tanks at approximately 2 l/min/tank, then was drained to the reservoir. In laboratory recirculation systems similar to those described above, Beard *et al.* successfully bred *P. merguiensis* through three generations (Beard *et al.*, 1977). Similar results were also reported for the breeding of *P. monodon* in laboratory recirculation systems (Beard and Wickins, 1980). Reid and Arnold reported that the production of *L. vannamei* could reach 11 kg/t when using ozone for water treatment in recirculating-water raceway systems (Reid and Arnold, 1994). Similar results were also obtained by Lin *et al* (2000a,b). Although considerable attention has been paid to the intensive culture of penaeid shrimp in indoor recirculation systems (Chen *et al.*, 2000; Lin and Tzeng, 1998, 2000; Lin *et al.*, 1998, 2000c,d,e; Ogle and Lotz, 2001), the techniques, especially in the spatial usage of the entire volume of water for culturing shrimp with their benthic nature, have rarely been developed, and the information of concern is limited.

Litopenaeus vannamei has been a popular cultured penaeid in Taiwan since 1994, and it has also been demonstrated to be a suitable species for indoor culture. The yields per ton of water reach reaching 5.2 and 11.5 kg in 75 and 105 d, respectively (Lin *et al.*, 2000a,b). Furthermore, fed with the same commercial pellets, *L. vannamei* had better food conversion and subsequently lower feeding costs than *P. monodon* (Lin *et al.*, 2000b). These all indicate that *L. vannamei* is a high-potential candidate for large-scale indoor production in the future. A previous study (Lin and Yeh, 2001) demonstrated that multi-layer net cages extended the spatial usage of water for growing *P. monodon* vertically. Total production increased in proportion to the number of layers in the cage; e.g., production rates in double- and triple-layer net cages were 2 and 2.5 times that in a single-layer one, respectively. Therefore, the present study aimed to increase the number of layers in a net cage to six and culture a fast-growing penaeid, *L. vannamei*, for higher production.

Materials and methods

This study was conducted in a greenhouse in the Tainan Mariculture Research Center, a branch of Taiwan Fisheries Research Institute. It lasted 57 days, from July 6 to September 19, 2001.

1.Shrimp Holding Facility

The cage we used was 1×1×0.9 m (L×W×H), which was composed of wooden frames and nylon nets of 1-mm mesh. The entire cage was supported by four 10-cm wooden feet at the four corners of the bottom. Bricks was put on the top of each cage to prevent it from floating and to ensure that the entire cage remained completely submerged in water. Layers of nylon net were secured horizontally in a cage. Distances between layers were 45, 30, and 15 cm for double-, triple-, and hexa-layer cages, respectively.

Six cages in total (two double-, two triple- and two hexa-layer net cages) were set in a single 22-t RC pond (6 m long × 3.5 m wide × 1.2 m deep, water depth of 1 m). The experiment was carried out in a zero exchange indoor shrimp culture system consisting of a bio-purification pond, which was connected to the cultured pond with PVC pipes, and a pumping machine of 150 W/220 A. Water was recirculated at a water flow of 62 l/min. Aeration in the pond was provided by aerators.

2.Initial Stocking Density

Each layer was stocked with 64 individuals/m^2 of *L. vannamei* which originated from a nursery pond with a salinity of 25 ppt. The average initial body weight was 0.15 g. The total numbers of shrimp stocked for each duble-, triple-, and hexa-layer net cage were 64 × 2, 64 × 3, and 64 × 6 individuals, respectively.

3.Analysis of Water Quality

Shrimp were cultured at room temperature, and water salinity was maintained at a designated level (25 ± 1 ppt) throughout the experiment. Water tempature, dissolved oxygen (DO), and pH were determined twice a week. Ammonia-N and nitrite-N were measured weekly using Merck kits.

4.Feeding Regimes

Shrimp were fed *P. monodon* commercial pellets containg 38% protein twice daily form Monday to Friday and rationed to once daily on Saturday and Sunday. The amount of feed per meal was 15% of the initial stocked biomass. The feed supply was not adjusted during the period of the experiment. Fifty-one tilapia individuals were placed outside the cages to consume any missed feed.

5.Caculation of Data

At the end of the experiement, shrimp were totally harvestd layer by layer to calculate the unit production and weight gain, and to count individuals for calculating the survival rate. The body weights of 20 individuals in each layer were randomly sampled for calculating the growth rate. The growth rate (GR) in terms of wet weight and the feed conversion ratio (FCR) were caculated as follows:

GR (g/d) = (W_2 - W_1) ÷ total stocking days, and

FCR = FI ÷ WG;

Where W_2 and W_1 are the final and initial wet body weight (g), and FI and WG are total food intake and total weight gain, respectively.

6.Statistical Analysis

Experimental data were analyzed with one-way ANOVA followed by Duncan's multiple range test. The significance level was set at 5%.

Results

During the cultur, the conditions of water (25 ± 1 ppt) were maitained at a water temperature of 30.6°C, pH of 8.13, DO of 7.29 ppm, ammonia-N of 0.3 ppm, and nitrite-N of 0.13 ppm on average (Table 1). It is very possible that the water quality within the cages differed from that outside the cages; data from both within and outside the cages were taken and compared during the experiment. For a triple-layer cage, all parameters except DO and pH, had almost the same values on average. DO in the uppermost layer was slight higher (8.10 ppm) than those of the middle layer , the bottom layer and the surrounding water . pH outside the cage, 8.07, was slight higher than readings within the cage, but there were no significant difference ($p > 0.05$) among them. The remaining results are described as follows.

1. Survival Rate

Average survival rates of double-, triple-, and hexa-layer cages were 80.5%, 73.4% and 75.9%, respectively (Table 2). No significant differences existed among them ($p > 0.05$).

By layers, there were similar trends among the three types of cages: the closer to the water surface the higher the survival rate. Differences between the topmost and bottommost were 10.9%, 3.9%, and 10.2% respectively on average for double-, triple- and hexa- layer cages (Table 3).

2. Growth Rate

Average growth rate of double-cages was 0.13, slight higher than those of triple-, and hexa-layer cages (Table 4). There were no significant differences among them ($p > 0.05$).

3. Unit Body Weight Gain in Each Layer of the Cages

The unit body weight gains which exceeded 345 g/m^2 in a single layer appeared in the third layer of the hexa-layer cage, both layers of the double-layer cage, and the topmost layer of the triple-layer cage. However, differences among all layers of the three types of cages did not reach a significant level ($p > 0.05$, Table 5).

4. Total Production

For an entire cage, after 57 d of culture, the total weight gain and total production were positively correlated with the number of layers. Comparatively, the average total production of a hexa-layer cage was significantly higher than those of the triple- and double-layer ones ($p < 0.05$, Table 6).

5. Harvest Sizes of Shrimp

Percentages of large- and small-sized shrimp for the double-layer cage were 72% and 28%, for triple-layer cages were 82% and 18%, and for hexa-layer cage were 83% and 17%, respectively. That is, the percentage of large-sized shrimp at harvest increased with a greater number of layers (Table 7). Body weights (g) of shrimp at harvest (footnote of Table 7) were 7.6 for the double-layer cage, 6.5 for the triple-layer cage, and 6.7 for the hexa-layer cage. Values did not significantly differ ($p > 0.05$).

6. Feed Conversion Ratio (FCR)

The FCR was less than 0.5 for any layer of the cages, and there were no significant differences among layers ($p > 0.05$, Table 8). The lowest FCR in the double-layer cage was the bottom (0.33), in the triple-layer cage was the top (0.36), and in hexa-layer cage was the third from the top (0.42). Values of FCR for an entire cage obtained from double-, triple-, and hexa-layer cage were 0.36, 0.41, and 0.44 respectively, and there were also no significant differences among them ($p > 0.05$, Table 9).

7. Labor Required for Feeding and Harvesting

It took 1, 1.5, and 3 min to feed shrimp manually through the feeding pipes for a double-, triple-, and hexa-layer cage, respectively. It took more than 1 h to complete the harvesting procedures for a hexa-layer cage.

Table 1.Water quality of the pond in which cages were placed for raising *Litopenaeus vanname*. Salinity: 25 ± 1ppt

Water temperature (°C)	pH	Dissolved oxygen (ppm)	Ammonia-N (ppm)	Nitriate-N (ppm)
30.6 ± 0.2	8.13 ± 0.01	7.29 ± 0.05	0.30 ± 0.06	0.13 ± 0.02

Data in all tables are given as the mean ± SE.

Table 2. Survival rate (%) in various types of cages

Double-layer	Triple-layer	Hexa-layer
80.47 ±6.26	73.44 ±2.35	75.91 ±1.47

Table 3. Survival rates (%) in each layer for various types of cages

1st	2nd	3rd	4th	5th	6th
		(From top to bottom)			
		Double-layer cage			
85.94 ± 4.68	75.00 ± 3.12				
		Triple-layer cage			
76.57 ± 7.81	71.10 ± 5.46	72.66 ± 0.78			
		Hexa-layer cage			
81.25 ± 1.56	76.57 ± 1.56	77.35 ± 2.35	75.00 ± 1.56	74.24 ± 7.03	71.10 ± 0.50

Table 4. Growth rates in Entire Cages

Double-layer	Triple-layer	Hexa-layer
0.13±0.02	0.12 ±0.00	0.12 ±0.00

Table 5. Weight gain (g/m^2) in each layer for various types of cages

1st	2nd	3rd	4th	5th	6th
		(From top to bottom)			
		Double-layer cage			
377.68 ± 0.47	447.76 ± 4.19				
		Triple-layer cage			
406.28 ±1.99	342.75 ± 28.09	343.34 ± 21.59			
		Hexa-layer cage			
340.17 ± 9.21	339.21 ± 10.19	345.22 ± 17.02	335.01 ±6.11	316.13 ±25.94	336.4 ±24.69

Table 6. Production (g/m^2) of entire cages for various types of cages

Double-layer	Triple-layer	Hexa-layer
824 ±45 ^{a}	1091±62 ^{a}	2014±19 ^{b}

Values with the same superscript letter do not significantly differ ($p > 0.05$).

Table 7. Percentage (%) and body weight (g) of large- and small-sized shrimp at harvest.

Size	Double-layer	Triple-layer	Hexa-layer
Large-size			
Percentage (%)	71.91 ± 3.40	81.83 ± 2.68	83.04 ± 2.49
Body Weight (g)	8.94 ± 0.54	7.74 ± 0.37	7.44 ± 0.20
Small-size			
Percentage (%)	28.09 ± 3.40	18.17 ± 2.68	16.96 ± 2.39
Body Weight (g)	3.18 ± 0.24	3.00 ± 0.12	2.99 ± 0.10

Table 8. Feed conversion rate (FCR) of each layer.

1st	2nd	3rd	4th	5th	6th
		(From top to bottom)			
		Double-layer cage			
0.39 ± 0.04	0.33 ± 0.01				
		Triple-layer cage			
0.36 ± 0.01	0.43 ± 0.04	0.43 ± 0.03			
		Hexa-layer cage			
0.45 ± 0.14	0.43 ± 0.01	0.42 ± 0.02	0.44 ± 0.01	0.46 ± 0.04	0.44 ± 0.04

Table 9. Feed conversion rate (FCR) of entire cages for various types of cages.

Double-layer	Triple-layer	Hexa-layer
0.36±0.03	0.40±0.02	0.43±0.01

Discussion

1. Survival Rate

By layers, there were similar trends among the three types of cages: the closer to the water surface the higher the survival rate, which was consistent with the result of a previous report (Lin and Yeh, 2001), indicating that the efficiency of water circulation or exchange is a critical factor for the multi-cage culture of shrimp, especially in the bottom layer.

2. Growth Rate

Average growth rates among the double-, triple-, and hexa-layer cages did not significantly differ. This is contrary to the result contrast for *P. monodon* (Lin and Yeh, 2001). Lin *et al.* (2000c) found that significant differences in growth rates existed among single-), double-, and triple-layer cages of *P. monodon* culture. Thus, *L. vannamei* may be superior to *P. monodon* for grow-out culture in multi-layer cages. The reason for this difference between the present study and that for *P. monodon* is not clear. It could have resulted from species differences or different water conditions related to different culture systems. In addition, Paquotte *et al.* (1998) reported *L. vannamei* is almost exclusively produced on the American continent, from the southern U.S. to northern Peru and Brazil. Growth rates in ponds range between 0.6 and 1.2 g/wk (i.e., 0.09 and 0.17 g /d), and vary between 0.63 and 0.84 g/wk (i.e., 0.09 and 0.12 g/d) in floating cages within estuarine zones. While compared with traditional pond culture and trials on estuarine floating cage culture, the growth rates obtained in this test, i.e., 0.12-0.13 g/d, were within the normal range, even a little higher than that of floating cages. Thus, indoor multi-layer cages should have higher production capacities for shrimp rearing.

3. Unit Body Weight Gain in Each Layer of the Cages

The unit body weight gain among all layers of three types of cages did not significantly differ, and this was consistent with results of our previous report (Lin and Yeh, 2001).

4. Total Production

Lin *et al.* (2000a,b) reported that with initial stocking densities of 667 and 2167 individuals/m^3, a cubic meter of water could produce market-sized shrimp of 5.2 and 11.5 kg with a duration of 75 and 105 d, respectively. In this study, the stocking density was 64 individuals/m^2 per layer (equal to 427 individuals/m^3), and the duration of culture was only 57 d. Elevation of the initial stocking density and prolonging culture duration over conditions evaluated in this study could promote production. Nevertheless, the average unit production of a hexa-layer cage was 2014 g/m^2, It apparently was higher than that of a floating cage (800 g/m^2)(Paquotte *et al.*, 1998), indicating that the multi-layer cage has high potential for the spatial usage of the entire volume of water for shrimp rearing. Furthermore, the hexa-layer net cage was the best for spatial usage of the entire volume of water, with a production 1.33 and 2.44 times that of the triple- and double-layer ones, separately (calculated from Table 6).

5. Harvest Sizes

Percentages of large-sized shrimp in hexa-layer (83%) and triple-layer cages (82%) were higher than that of the double-layer cage (72%), suggesting that decreases in space due to an increase in the number of layers in a cage with a constant volume greatly benefited the increasing percentage of large-sized shrimp at harvest. In a 0.9-m^3 volume cage of the present study, the layer volumes of double-, triple-, and hexa-layer cages were 0.45, 0.30, and 0.15 m^3, respectively.

6. Feed Conversion Ratio (FCR)

Differences between the maximum and minimum FCR values were 0.11, 0.11, and 0.07 for the double-, triple- and hexa-layer cages, respectively. In other words, differences in the hexa-layer cage were found to be the smallest. This agrees with SE values obtained from various types of cages (hexa-layer, 0.01; double-layer 0.03; triple-layer, 0.18, Table 9). This implies that the FCR could be stabilized by decreasing the space (i.e., increasing the number of layers).

In indoor shrimp culture using the same brand of *P. monodon* commercial pellet food to feed *L. vannamei*, *Metapenaeus ensis*, *P. chinensis* and *P. monodon* at the same stocking density of 500 individuals/m^2, FCR values were 0.89, 1.45, 2.60, and 2.90, respectively (Lin et al., 2000a). This suggests that *P. monodon* commercial pellet manufactured in Taiwan is a suitable diet for *L. vannamei*, with a low feeding impact on the environment.

In the present study, the value of FCR was lowed to less than 0.5. The reason for this notable result was due to the design feeding regime. Shrimp were fed twice daily from Monday to Friday and then rationed to once a day on Saturday and Sunday. The amount of feed per meal was 15% of the initial stocked biomass, and the feed supply was not adjusted during the period of the experiment.

From the harvested size and survival rate, the decline in the amount of feed as expressed in the percentage of total present biomass of shrimp in terms of the feeding rate could be estimated. On the 57th day at the end of experiment, the feeding rate was estimated to be only 0.85%, which is not sufficient according to my knowledge. Thus the shrimp might have been consuming other things such as shrimp caracasses, newly molt shrimp, and other organisms from the water and substratum (nets) as supplemental food.

The average survival rate of shrimp in hexa-layer cage was 76%. This indicates that at least 15 shrimp individuals [64 – (64 × 76%) = 15.36] of each layer were possibly sacrificed for food intake during the period of culture.

The survival rate was apparently lower as compared with that of a previous study (Lin et al., 2000a), suggesting that the survival rate of the present study would have been improved by adjusting the amount of pellets daily or at regular intervals after stocking as was done in previous studies (Lin *et al.*, 2000a,b).

At harvest, no apparent organic debris was found in any layer of the cages. The nets of the cages were almost as clean as they were originally. This was contrary to the situation in a previous study on *P. monodon* (Lin *et al.*, 2000c), indicating that *L. vannamei* may be more omnivorous compared with *P. monodon* in captivity.

Wilcox and Jeffries reported that an ability to utilize diverse foods has survival value in a seasonally changing environment (Wilcox and Jeffries, 1974). Thus, when a preferred food is scarce, organic debris is usually available to estuarine animals. In the present study, food intake was restricted, and according to our observations, *L. vannamei* browsed on microorganisms which grew on the substratum, and mud particles were always found in the alimentary canal of this species before food intake or on the days of fasting treatment. Furthermore, in both RC ponds and FRP tanks in which *L. vannamei* was stocked in high densities (Lin *et al.*, 2000a,b), few microorganisms deposited animal feces on the bottom. Therefore, we speculated that *L. vannamei* was consuming the fecal material. In a study by Wilcox and Jeffries (1974), they found that seven crustaceans ate fecal material. Bartlett *et al.* (1993) cultured *L. vannamei* without offering any feed in cages of plastic netting to test the possibilities of reducing food costs on shrimp farms by using plastic netting as a surface for growth of natural food. Seven different experiments were performed in which shrimp were grown at densities ranging from 6 to 40 shrimp per square cage for periods ranging from 5 to 14 weeks, resulting in growth rates of 0.5 to 1.9 g/wk. They concluded that under favorable conditions, the cage micro-ecosystem is capable of supporting growth rates of over 1.0 g/wk at densities above 10 shrimp and with a biomass of up to 200 g/m^2. This supports our speculation mentioned above. In addition, according to Akiyama *et al.* (1989) there were no differences in apparent protein digestibility due to animal or plant feedstuff origin for *L. vannamei*. Collectively, the feeding regime of the present study resulted in the lower value of FCR for indoor *L. vannamei* cage culture.

7. Labor Required for Feeding and Harvesting

Harvesting and feeding appear to be two potential problems for multi-layered shrimp culture; in particular, it took more than 1 h to complete the harvesting procedures for a hexa-layer cage. Furthermore, during the culture period, it was also very difficult to sample the shrimp from cages; therefore, the construction of multi-layered cages needs to be improved for easier feeding, sampling, and harvesting purposes.

Conclusions and suggestions

Hexa-layer cages can stock more shrimp as well as obtain higher production values compared with either triple- or double-layer cages, and for indoor cage culture, there is no problem of shrimp being lost in storms which has occurred in estuaries (Shanmugam *et al.*, 1995), benefiting the development of indoor shrimp farming in the future.

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二、三及六層箱網室內養殖白蝦的效果比較

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摘要

本研究針對三種立體箱網在室內養殖白蝦的活存與成長加以比較。放養密度爲 64 尾/米平方，共計養殖 57 天，鹽分維持在 25 ppt，水溫 29.1-31.6°C，最初体型爲 0.15 公克。同體積二、三及六層箱網養殖白蝦的結果以六層箱網最佳。結果顯示六層箱網在水體的充分利用上較優，其總產量爲三層箱網及二層箱網的 1.3 倍及 2.4 倍。六層箱網的各層平均活存率爲 75.9%，三層箱網者爲 73.4%，二層箱網者爲 80.5，統計上無差異($p > 0.05$)，收成的體型三者間亦無差異($p > 0.05$)，但大型蝦的百分比以六層箱網者爲最高。餌料係數在本研究的結果低於 0.5, 此值得注目的結果在文中有詳加討論。

關鍵詞：多層箱網　活存率　成長率　單位產量　餌料係數