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# Efficacy of Probiotics and Bioflocs on the Production Performance of Tiger Shrimp *Penaeus monodon* and Pacific White Shrimp *Litopenaeus vannamei* during Polyculture Operation

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors KS and YA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript' managed the analyses of the study, managed the literature searches. Author MSR conceived and designed the study. All authors read and approved the final manuscript.*

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## ABSTRACT

Shrimp culture has been developed during the last three decades and the production of farmed shrimp reached its peak. However, the shrimp production was decreased all over the World including in Asian Countries because of the mass mortality due to the outbreak of several diseases predominantly White Spot Syndrome Virus (WSSV) which caused extensive economic damage to the shrimp culture industry. Various Polyculture technologies of shrimp with shellfish, finfish or seaweeds have been implemented to reduce economic damages by mass mortality of shrimp. The present study was conducted with Tiger shrimp *Penaeus monodon* and Pacific white shrimp *Litopenaeus vannamei* through Polyculture operation to determine optimum stocking ratios for induction of best performance and highest production rates. Initially, the experiments were conducted with different ratios and combinations of stocking densities 1:1, 2:1 and 1:2 ratios of *Penaeus monodon* and *Litopenaeus vannamei* with control group feed with commercial feed, the other group certain Probiotic bacteria such as *Bacillus licheniformis* and *Lactobacillus rhamnosus* added in feed, the third group both the Probiotic bacteria and the addition of external Carbon

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source from sugarcane molasses in the form of Bioflocs for 100 days. To ascertain further interaction of ratios influence, 20:10, 20:12, 20:15 ratios of stocking and Monoculture experiments were conducted. In both the experiments the Survival rate, Final body weights, Weight Gain, Feed conversion ratio, Specific growth rates, Average daily growth rates, Protein efficiency ratio, and Productivity rates were significantly different (P<0.05) in all the different treatments both for *P. monodon* and *L. vannamei*. From the results obtained 20:10 ratio of *P. monodon* and *L. vannamei* was considered as the best ratio of stocking, which yielded the highest production rates in all the Control, Probiotic added and both Probiotic & Biofloc added groups. The results obtained in the present study clearly demonstrate that the rearing of taxonomically similar species with optimum stocking ratios seems to improve the efficiency of shrimp farming and substantially increasing the production rates. So, this polyculture of *P. monodon* and *L. vannamei* can be considered as an alternative approach towards the establishment of sustainable shrimp farming activity which will yield good economic returns.

*Keywords: Penaeus monodon; Litopenaeus vannamei; probiotics; bioflocs.*

## 1. INTRODUCTION

Shrimp farming is one of the fastest-growing components of the global aquaculture industry. Most of the farmed shrimp production is in Asia (78%) with tiger shrimp *Penaeus monodon* was the most important culture species, accounting for 58% of the cultured shrimp production, followed by white shrimp *Litopenaeus vannamei* at 20% [1]. Although shrimp farming has been quite successful and the technology has continually improved, it has caused some environmental damage such as eutrophication, sedimentation, in Coastal areas, chemical bioaccumulation in the water-borne and deleterious pond bottom soil. Despite severe problems rose in the shrimp farming industry, the World demand relatively high which draws high attention to the sustainable aquaculture system for recovering production. A sustainable aquaculture system requires consideration of environmental soundness, economic return that contributes to the marginal rate for investment. The primary goal of rational pond management is to utilize existing conditions in the ponds to produce fish to maximize economic returns to the entrepreneur. However, the Polyculture is one of the sustainable aquaculture systems that offer the desired level of production and raises both the culture profit and ecological efficiency. The main concept of Polyculture is based on the fact that the rearing of two or more compatible species together will result in higher production compared to monoculture [2]. Polyculture utilizes the concept that a mixed stock of selected fish or shrimp species, with complementary or minimal competing feeding habits and different ecological requirements, can exploit the resources of different ecological niches in a pond efficiently, thereby resulting in maximum fish production for given input quantities. Stocking two or more Complementary species can increase the maximum standing crop of a pond by allowing a wider range of available foods and pond volume to be utilized [3] in several maritime countries polyculture of fish and other marine and brackish water shrimps has been conducted. In the past decade, studies about the Crustacean polyculture have increased, demonstrating in some cases that it is not only profitable, but also a sustainable activity, both in freshwater and marine systems [4]. Integrated culture is considered as an effective strategy to minimize waste in the farm system. Several benefits have been reported in shrimp polyculture systems when using fish and other aquatic organisms as subordinate species, although polyculture is not yet a common practice [5]. Moreover, several authors believed that adding secondary species improves the performance of the main culture organism [6]. Belton and Little [7] demonstrated that integrated culture practices, such as polyculture are good alternatives for reducing contamination also, it can contribute to minimizing the environmental impact of farm effluents, particularly those related to nitrogenous wastes, which are further converted into toxic metabolites. The main reason for this is that some subordinate species can feed on and assimilate most of the wastes generated from shrimp aquaculture. Therefore, the present investigation on feasibility of tiger shrimp *P. monodon* with pacific shrimp *L. vannamei* mixed culture is interesting to conduct. This study would evaluate the optimum stocking performance to obtain maximum productivity. The results obtained from this study will pave way for assessing the relative production efficiency during mixed culture operation.

## 2. MATERIALS AND METHODS

The present study was carried out in shrimp culture farms located in Ramayapatnam (Latitude $15^{0}$ $02^{1}$ $55^{11}$ N; Longitude $80^{0}$ $02^{1}$ $50^{11}$ E) Prakasam Dist of Andhra Pradesh, India. The culture ponds selected in the present study are approximate with an average size of 1.0-1.2 ha and are rectangular in shape and soil is ideal for semi-intensive type of culture operation. The ponds were first dried and exposed to sunlight before the beginning of the experiment. Lime (CaO) was applied to the pond bottom at 500 kg/ha. After 9 weeks of lime application, ponds were filled with already stored brackish water in the stocking pond. Water for culture operation was drawn with the help of motors from Buckingham canal and kept in a storage pond for further use in the culture operation. The water depth in all the culture ponds was kept at 50-60 cms and after 10 days the ponds were fertilized with organic manure, cattle dung, and inorganic fertilizers, urea, and single super phosphate at 2000, 50, and 50 kg/ha, respectively. Then all the ponds were allowed for again one week to ensure the growth of natural fish food organisms and water level was finally raised to110-120 cms.

Shrimp *P. monodon* and *L. vannamei* of 0.65±0.03 g of uniform size were obtained from local aquafarms and pathogen-free shrimp were selected and acclimatized in the experimental tanks with a salinity of 10±0.5 ppt. Feeding was done with a commercial feed obtained from the local market 3-6% body weight per day. The daily ration was distributed in two equal quantities in the morning (06.00 hrs) and afternoon (18.00 hrs). The feed was provided in feed trays and also broadcasted into the culture pond. Initially crumbled feed was given, as the culture progressed the pellet size was increased to fit changes in shrimp sizes. Feed quantity was adjusted every 15 days based on the shrimp body weights calculated from periodical sampling and also assumed survival percentages at different stages of culture operation. During this experiment, no water exchange was done, but a water level of 110-120 cms was maintained throughout the culture period by the addition of water drawn from treated stocking ponds. During the culture operation maintenance of pH was collected fortnightly with the application of limestone powder at 200 kg/ha. Cattle dung, urea, and single super phosphate were applied at 200, 20, and 20 kg/ha respectively, at 20 day interval to maintain sustained desirable water quality in conjunction with optimum natural productivity. The experiment was conducted for 100 days.

### 2.1 Probiotic Feed Preparation

Probiotic supplemented feeds were prepared as described by Naresh [8]. Probiotic bacterial species *Bacillus licheniformis* and *Lactobacillus rhamnosus* were obtained and maintained in the nutrient broth and were harvested by centrifuging at 10,000 rpm for 10 minutes, subsequently washed with phosphate buffer, finally re-suspended in phosphate buffer saline (pH 7.4). These re-suspended bacteria were mixed uniformly to the feed pellets by the spraying method. The Probiotic blended feed prepared was dried at $40^{0}$C and packed in air-tight polythene covers and stored in a Refrigerator for further use. The Probiotic blended feed with *L. rhamnorus* and *B. licheniformis* @ 10 billion cfu/kg feed was prepared once in seven days.

### 2.2 Preparation of Biofloc

Sugarcane molasses was selected as a source for Carbon and known to contains 36% Carbon, 53% Carbohydrate, 24% moisture content was incubated for 2 days in warm water at $40^{0}$C, and the same was added to the culture medium in the ratio 1:3 Molasses: Water. To stimulate Nitrogen loading in an aquaculture system, $NH_4Cl$, $KH_2PO_4$, and $Na_2HPO_4$ were added to each tank @ 96, 31, and 64 mg/lit, respectively. The ratio between sugarcane molasses and feed to reach the desired Carbon: Nitrogen (C:N) ratio was calculated based on assuming 50% nitrogen from feed eaten by the shrimp excreting into the water environment [9]. On the above basis the formula of the ratio in weight between, the Carbon source and feed can be given as follows

$$\Delta CH / \Delta F = ((CN \times \% P(F) \times \% N(P)) - \% CF / \% C_{CH}$$

Where,

$\Delta CH$ : Weight of Carbon Source
$\Delta F$ : Weight of the Feed
CN : C:N ratio need to be required
% P(F) : Protein content in Feed
%N(P) :Nitrogen content in Protein (15.5 %)
% CF : Carbon content in the Feed (50%)
$\% C_{CH}$ :Carbon content in the Carbon Source

Carbon content was determined by adopting the method of Walkley and Black [10]. Total Ammonia Nitrogen (TAN) concentration and other water quality parameters were measured with the procedures according to APHA [11]. Growth parameters including, average body weights, average body growth rates, specific growth rates, feed conversion ratio, protein efficiency ratio, feed efficiency ratio and productivity rates were monitored and tabulated. All the above parameters were calculated by adopting the following formulae:

Survival rate (%) = (Total number of live shrimp / Total number of shrimps stocked) X 100

Weight Gain (g) = Weight of the shrimp (g) at the end of the expt. - Weight of the shrimp (g) at the start of the expt.

Feed Conversion Ratio (FCR) = (Total amount of feed Consumed (Kgs) / Total biomass of shrimp (Kgs))X 100

Average daily growth rates (ADGR) = (Weight of the shrimp (g) at the end of the expt. - Weight of the shrimp (g) at the start of the expt.) / Total number of days of experiment X 100

Specific growth rates (SGR) = (Log weight of the shrimp (g) at the end of the expt. - Log weight of the shrimp (g) at the start of the expt.) / Total number of days of Experiment X 100

$$(\text{Log } W2 - \text{Log } W1) / T \times 100$$

Where,

W1 :Weight of the shrimp at start of the Experiment
W2 :Weight of the shrimp at the end of the Experiment
T :Total number of days of Experiment

### 2.3 Data Analysis

The data obtained were analyzed statistically through Microsoft excel. The difference invariants between treatments was determined according to One-Way ANOVA using SPSS. If there were significantly followed by Duncan's multiple range test.

## 3. RESULTS AND DISCUSSION

In the present investigation, an attempt has been made to study the performance of *P. monodon* and *L. vannamei* in monoculture, mixed culture at different stocking densities to assess the production rates after 100 day experiment. In the present study, all the experimental ponds were stocked with 40,000 Nos of *P. monodon* or *L. vannamei* either individually or in mixed proportion. The Harvest data obtained in the present study was presented i.e. for control pond (Table 1), for Probiotic added pond (Table 2), for Probiotic and Biofloc added pond (Table 3). In the present study initially, ratios of 10:10, 20:10, and 10:20 of *P. monodon* and *L. vannamei* in the total stocking density of 40,000 Nos was stocked and the details of stocking nos were presented in Tables 1-3. Based on the results obtained, a ratio of 20:10 *P. monodon* and *L. vannamei* mixed culture obtained highest and best growth potentials, further ratios of 20:12 and 20:15 of *P. monodon* and *L. vannamei* and also monoculture was conducted for 100 days to assess and arrive at the precise rate of optimal stocking density to maximize the productivity rates (Table 4) (Figs.1-3). Consolidated harvest details of shrimp *P. monodon* and *L. vannamei* uptained in the present study were presented in Table. 5 under different experimental conditions. Among the three ratios of 10:10, 20:10, and 10:20 of *P. monodon* and *L. vannamei* selected, 20:10 ratio yielded best and highest growth rates after 100 day experimental period. From the results obtained, the highest and best growths were recorded with Probiotic and Biofloc added group, followed by Probiotics added group compared to control group, fed with only commercial feed for a period of 100 days. In every group of experiment, 40,000 Nos of both put together *P. monodon* and *L. vannamei* were stocked. The percent survival values recorded were found to be in the range between 64-69% for 10:10, and 10:20 ratio groups compared to 20:10 ratio group, which is slightly higher ranges 75-76% and also found to be significant ($P<0.05$) in the control group. After the completion of 100-day experimental period in the control group recorded average body weights of 27.69, 29.85 and 26:88 g for 10:10, 20:10 and 10:20 ratio groups and found to be significant ($P<0.05$). But for *P. monodon* recorded 29.13, 30.76 and 28.04 g and for *L. vannamei* 26.24, 28.94 and 25.72 for 10:10, 20:10 and 10:20 ratio groups and found to be significant ($P<0.05$). The average Specific growth rates (SGR) obtained were 2.08, 1.94 and 1.90 for 10:10, 20:10 and 10:20 ratios, respectively and found to be significant ($P<0.05$). The average daily growth rates (ADGR) obtained in the present study were 0.271, 0.292 and 0.263 g/day for 10:10, 20:10 and 10:20 ratios, respectively and were found to be significant ($P<0.05$). The Feed Conversion Ratio (FCR) values were obtained were found to be 2.79, 2.05 and 2.58 for 10:10, 20:10 and 10:20 ratios, respectively and found to be significant (P<0.05). Feed Efficiency (FE) percentages obtained were 36, 49 and 39 for 10:10, 20:10 and 10:20 ratios, respectively and found to be significant (P<0.05). Protein Efficiency Ratio (PER) values recorded in the present study were 5.39, 6.32 and 6.08 for 10:10, 20:10 and 10:20 ratios, respectively and found to be significant (P<0.05). The total productivity values obtained in the present study were recorded as 697, 892 and 709 kgs for 10:10, 20:10 and 10:20 ratio groups. Yield ratios recorded as 1.13, 2.12 and 0.55 for production rates for *P. monodon* and *L. vannamei* in the present study at 10:10, 20:10 and 10:20 ratios, respectively. The performance details of *P. monodon* and *L. vannamei* after the Probiotics added to the feed were also monitored and presented in Table 2. The average percent survival values recorded to be 72.5, 84.5 and 73.5% for 10:10, 20:10 and 10:20 ratios, respectively. The average final body weights obtained in the present study were 31.64, 33.81 and 29.69 g for 10:10, 20:10 and 10:20 ratios, respectively and were found to be significant (P<0.05). The average increments in the growth rates were 4767, 5102 and 4467% for 10:10, 20:10 and 10:20 ratios, respectively and were found to be significant (P<0.05). The average daily growth rates (ADGR) obtained in the present study were 0.310, 0.332 and 0.291 g/day for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). Similarly, SGR values were recorded as 1.97, 2.00 and 1.94 for 10:10, 20:10 and 10:20 ratios of stocking densities, respectively. DGR values were recorded as 0.310, 0.332 and 0.291 for 10:10, 20:10 and 10:20 ratios of stocking densities, respectively and were found to be significant (P<0.05). The FCR values were found to be 2.36, 1.84 and 2.18 for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). FE values were recorded as 42, 54 and 46% for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). PER values obtained were 7.04, 7.59 and 7.12 for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). Similarly, Productivity values obtained were 450, 562 and 422 kgs for 10:10, 20:10 and 10:20 ratios for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). The average productivity values obtained were 899, 1124 and 844 kgs/crop for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). The performance details of *P. monodon* and *L. vannamei* after both Probiotics and Bioflocs added into the culture operation were monitored and presented in Table 3. The percent survival values recorded as 84.5, 92 and 80% for 10:10, 20:10 and 10:20 ratios of stocking, respectively. The average final body weights were also found to be 37.28, 38.31 and 35.53 g for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). The average increment growth rates were found to be 5635, 5794 and 5366% for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). The ADGR values were found to be 0.366, 0.377 and 0.349 g for 10:10, 20:10 and 10:20 ratios of stocking, respectively and were found to be significant (P<0.05). The FE, PER and ASGR values obtained were found to be 50, 7.49, 2.05 for 10:10 ratio of stocking, 61, 8.45, 2.05 for 20:10 ratio of stocking, and 52, 7.74 and 2.0 for 10:20 ratio of stocking and were found to be significant (P<0.05). The average Productivity and Total Productivity, and yield ratios were recorded as 620, 1239 and 1.16 for 10:10 ratio of stocking, 702, 1404, 2.14 for 20:10 ratio of stocking, 547, 1094, 0.574 for 10:20 ratio of stocking groups and were found to be significant (P<0.05).

After obtaining results from the first set of experiment with stocking ratios of 10:10, 20:10 and 10:20, another set of experiment was designed to study and monitor the growth potentials in the stocking ratios of 20:12, 20:15 and also in monoculture of *P. monodon* and *L. vannamei* for 100 days. In the present set experiment was conducted with both Probiotics and Bioflocs were added into the culture operation showed an survival rates more than 85 and a maximum of 88%. An average of final weights of 35.13, 34.43 and 37.40 g for 20:12, 20:15 ratios of stocking and Monoculture operation, respectively and were found to be significant (P<0.05). The increment in the weight gain were found to be 5304, 5197 and 5653% for 20:12, 20:15 ratios of stocking and Monoculture operation and were found to be significant (P<0.05). The average Productivity and Total Productivity rates were recorded as 604, 612, 628 kgs and 1208, 1224 and 1256 kgs for 20:12, 20:15 ratios of stocking and Monoculture operation, respectively. Average daily growth rates 0.345, 0.338 and 0.368, Feed Conversion Ratio (FCR) 1.82, 1.88 and 2.34, and Feed Efficiency (FE) 55, 53 and 43% recorded with 20:12, 20:15 and Monoculture operation, respectively (Table 4). Consolidated statement of performance details of *P. monodon* and *L. vannamei* obtained under different stocking ratios and treatments were presented in Table 5. From the results obtained it is very clear that maximum growth rates and production rates were recorded with the experimental group in which both Probiotics and Bioflocs added into the culture operation closely followed by Probiotics added experiment al group finally minimum growth rates obtained with control group. Among the stocking ratios maintained in the present investigation, 20:10 ratios of stocking yielded maximum production rates compared to other ratios of stocking including 10:10 and 10:20. In between the two species selected in the present study, *P. monodon* showed relatively more growth rates and production yield compared to the other species *L. vannamei*.

In the present study, direct polyculture or mixed culture system was adopted, mixing of two species in the same pond or aquaculture unit without partitioning. This type of polyculture does not require significant extra economical investment because the secondary species are simply added within the same space of the main species. Thus, there is no necessity for more space or additional investment. Increasing stocking density of *L. vannamei* had effects on survival rate, growth rates and finally production rates of *P. monodon* due to higher competition and much more aggressive nature of *L. vannamei*. The competition increased loss of species and was more intensified, largely as a result of direct competition for food particles. The results obtained in the present study gains support from earlier reports, that competition exists in polyculture is natural and will be more intensive between taxonomically similar species [12]. Several authors reported that growth rates, mean weights and total productivity were found to be significantly higher in polyculture of shrimp and fishes of different categories compared with monoculture system [2,13-16]. Integrated culture is considered as an effective strategy to minimize waste in the farm system. Several benefits have been reported in shrimp polyculture systems when using fish and other aquatic organisms as subordinate species, despite the fact that polyculture is not yet a common practice [5]. Moreover, several authors also believed that adding secondary species improves the performance of the main cultured organism [6, 17] and considered to be good alternative for reducing contamination also. It can contribute to minimizing the environmental impact of farm effluents, particularly those related to nitrogenous wastes, which are further converted into toxic metabolites. The main reason for this is that some subordinate species can feed on and assimilate most of the wastes generated from shrimp aquaculture. Several authors also reported that shrimp culture with mullet and milk fish had benefits in the removal of nitrogenous wastes from the shrimp ponds [18,19]. Generally, many benefits have been achieved in shrimp polyculture systems when using fish, bivalves and seaweeds. The benefits include the diminution of ecological impacts and maintenance of water quality which subsequently improve total biomass of shrimp [20]. The growth of shrimp is generally dependent on the feed availability and water quality [16,21]. In the present study, the source of feed for shrimp was distributed equally in all the experimental systems to the standard plan by following in accordance with, strict biomass theory. Thus, the better shrimp harvest from polyculture and also in probiotic and biofloc added groups from culture ponds probably return to factors other than artificial food, factors such as natural productivity and maintenance of ideal water quality culture ponds. Earlier several authors reported that due to addition of probiotics into the system plays an important role in minimizing the nitrogenous wastes, there by production of toxic substances were also substantially reduced followed by higher efficiency of nitrogen utilization in culture operation facilitates a consequent decrease in nitrogenous substances, thereby improving the water quality. Due to the addition of bioflocs in to the culture operation also facilitates the production of heterotrophic bacteria and planktonic materials in the culture system, which will acts as supplementary feeding materials in addition to the feeds broadcasted, induces the highest and best growth potentials in shrimp *L. vannamei* [22, 23]. In the present study, a unique attempt has been made to conduct a polyculture of two penaeid shrimp species i.e. *P. monodon* and *L. vannamei*, manipulation of stocking ratios reduced the inter-specific competition, thereby increased the survival rates followed by production rates. The present study revealed that stocking with taxonomically similar species with high density was possible by designing the ratio of density between the two species selected in the polyculture operation.

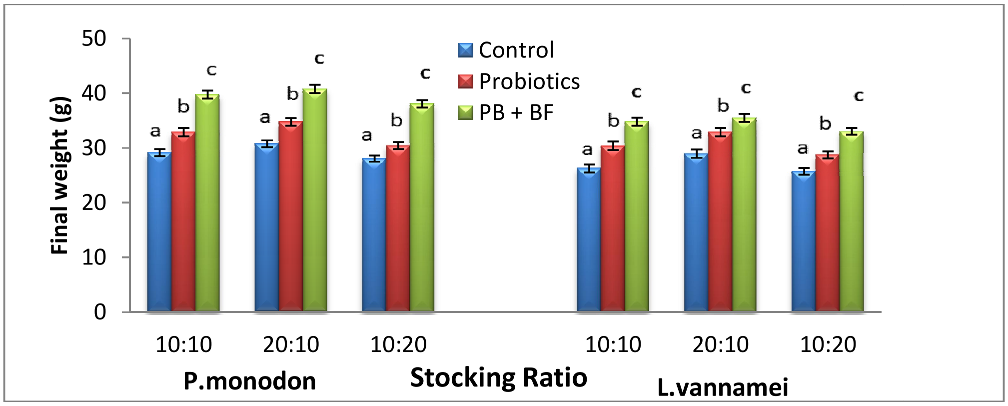


**Fig. 1. Final body weights of *P. monodon* and *L. vannamei* at different stocking ratios under various feeding trails**

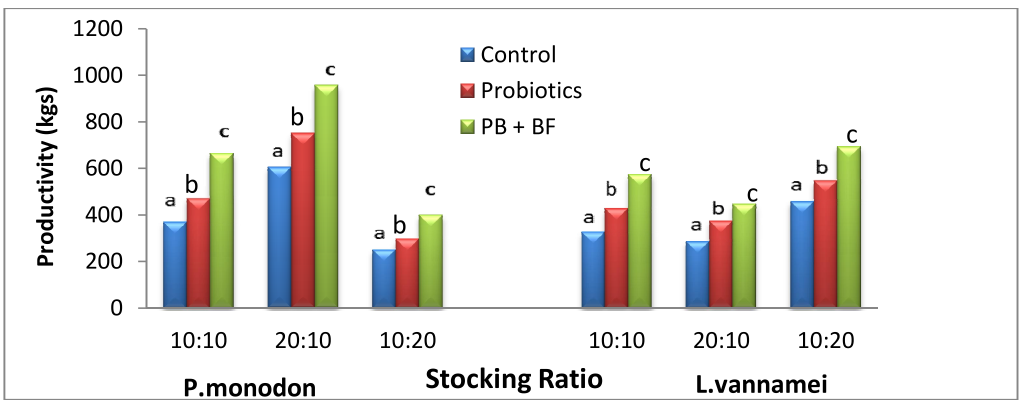


**Fig. 2. Productivity of *P. monodon* and *L. vannamei* at different stocking ratios under various feeding trails**

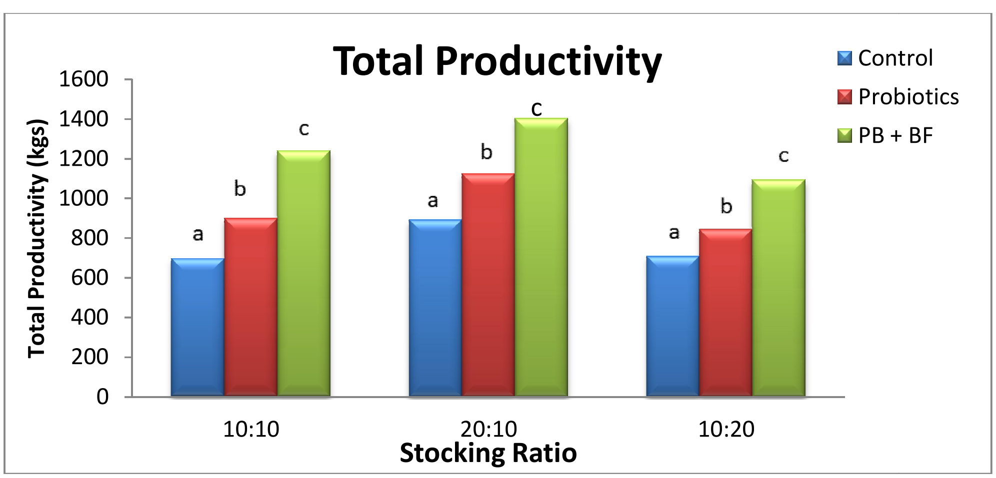


**Fig. 3. Productivity of shrimp at different stocking ratios under various feeding trails**

**Table 1. Performance details of *P. monodon* and *L. vannamei* under different experimental feeding trails at variable stocking densities under polyculture (control group)**

| Parameter | Stocking Ratio 10:10 | | Stocking Ratio 20:10 | | Stocking Ratio10:20 | |
|---|---|---|---|---|---|---|
| | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** |
| Number of shrimp stocked | 20000 | 20000 | 26500 | 13500 | 13500 | 26500 |
| Percent survival | 65 | 64 | 76 | 75 | 68 | 69 |
| Average Percent Survival | 64.5 | | 75.5 | | 68.5 | |
| Final weight (g) | 29.13 ± 0.64$^{a}$ | 26.24 ± 0.75$^{a}$ | 30.76 ± 0.63$^{a}$ | 28.94 ± 0.77$^{a}$ | 28.04 ± 0.57$^{a}$ | 25.72 ± 0.61$^{a}$ |
| Average Final Weight (g) | 27.69$^{b}$ | | 29.85$^{c}$ | | 26.88$^{d}$ | |
| Weight gain (g) | 28.48 ± 0.65$^{b}$ | 25.59 ± 0.73$^{c}$ | 30.11 ± 0.58$^{b}$ | 28.29 ± 0.59$^{c}$ | 27.39 ± 0.61$^{b}$ | 25.07 ± 0.58$^{c}$ |
| PDC | 4382$^{a}$ | 3937$^{a}$ | 4632$^{a}$ | 4352$^{a}$ | 4214$^{a}$ | 3857$^{a}$ |
| Average PDC | 4160$^{a}$ | | 4492$^{a}$ | | 4036$^{a}$ | |
| Specific Growth Rates (SGR) | 2.27$^{b}$ | 1.88$^{c}$ | 1.95$^{b}$ | 1.92$^{c}$ | 1.91$^{b}$ | 1.88$^{c}$ |
| Average SGR | 2.08$^{b}$ | | 1.94$^{c}$ | | 1.90$^{b}$ | |
| Daily Growth rates (DGR) (g) | 0.285 ±0.012$^{b}$ | 0.256 ± 0.010$^{c}$ | 0.301±0.013$^{b}$ | 0.283 ± 0.012$^{c}$ | 0.274 ±0.012$^{b}$ | 0.251±0.011$^{c}$ |
| Average DGR (g) | 0.27$^{b}$ | | 0.292$^{b}$ | | 0.263$^{d}$ | |
| Feed Conversion Ratio (FCR) | 2.79$^{b}$ | | 2.05$^{b}$ | | 2.58$^{d}$ | |
| Feed Efficiency (FE) (%) | 36$^{b}$ | | 49$^{b}$ | | 39$^{d}$ | |
| Protein Efficiency Ratio (PER) | 5.39$^{b}$ | | 6.32$^{c}$ | | 6.08$^{d}$ | |
| Productivity (kgs) | 370$^{b}$ | 327$^{c}$ | 606$^{b}$ | 286$^{c}$ | 251$^{b}$ | 458$^{c}$ |
| Average Productivity (kgs) | 349$^{b}$ | | 446$^{b}$ | | 354$^{d}$ | |
| Yield Ratio | 1.13$^{b}$ | | 2.12$^{b}$ | | 0.55$^{d}$ | |
| Total Productivity (kgs) | 697$^{b}$ | | 892$^{b}$ | | 709$^{d}$ | |

*Values are mean ± SD of six individual observations; Values are statistically Significant at $^{a}P<0.05$ from its respective controls; Means with different superscripts for each in a column differ significantly at P<0.05*

**Table 2. Performance details of *P. monodon* and *L. vannamei* under different experimental feeding trails at variable stockingdensities under polyculture (probiotics added)**

| Parameter | Stocking Ratio 10:10 | | Stocking Ratio 20:10 | | Stocking Ratio 10:20 | |
|---|---|---|---|---|---|---|
| | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** |
| Number of shrimp stocked | 20000 | 20000 | 26500 | 13500 | 13500 | 26500 |
| Percent survival | 73 | 72 | 83 | 86 | 74 | 73 |
| Average Percent Survival | 72.5 | | 84.5 | | 73.5 | |
| Final weight (g) | 32.89 ±0.75$^{a}$ | 30.38 ± 0.79$^{a}$ | 34.76±0.72$^{a}$ | 32.86 ±0.76$^{a}$ | 30.42 ±0.64$^{a}$ | 28.75 ± 0.64$^{a}$ |
| Average Final Weight (g) | 31.64$^{b}$ | | 33.81$^{c}$ | | 29.69$^{d}$ | |
| Weight gain (g) | 32.24±0.76$^{b}$ | 29.73 ± 0.71$^{c}$ | 34.11±0.68$^{b}$ | 32.21 ±0.69$^{c}$ | 29.77±0.62$^{b}$ | 28.30 ±0.62$^{c}$ |
| PDC | 4960$^{b}$ | 4574$^{c}$ | 5248$^{b}$ | 4955$^{c}$ | 4580$^{b}$ | 4354$^{c}$ |
| Average PDC | 4767$^{b}$ | | 5102$^{c}$ | | 4467$^{d}$ | |
| Specific Growth Rates (SGR) | 1.98 | 1.95 | 2.01 | 1.98 | 1.95 | 1.93 |
| Average SGR | 1.97$^{b}$ | | 2.00$^{b}$ | | 1.94$^{b}$ | |
| Daily Growth rates (DGR) (g) | 0.322 ±0.013$^{b}$ | 0.297 ±0.011$^{c}$ | 0.341 ±0.014$^{b}$ | 0.322 ±0.015$^{c}$ | 0.298 ±0.013$^{b}$ | 0.283 ±0.014$^{c}$ |
| Average DGR (g) | 0.310$^{b}$ | | 0.332$^{c}$ | | 0.291$^{d}$ | |
| Feed Conversion Ratio (FCR) | 2.36$^{b}$ | | 1.84$^{c}$ | | 2.18$^{d}$ | |
| Feed Efficiency (FE) (%) | 42$^{b}$ | | 54$^{c}$ | | 46$^{d}$ | |
| Protein Efficiency Ratio (PER) | 7.04$^{b}$ | | 7.59$^{c}$ | | 7.12$^{d}$ | |
| Productivity (kgs) | 471$^{b}$ | 428$^{c}$ | 750$^{b}$ | 374$^{c}$ | 297$^{b}$ | 547$^{c}$ |
| Average Productivity (kgs) | 450$^{b}$ | | 562$^{c}$ | | 422$^{d}$ | |
| Yield Ratio | 1.10$^{b}$ | | 2.01$^{c}$ | | 0.54$^{d}$ | |
| Total Productivity (kgs) | 899$^{b}$ | | 1124$^{c}$ | | 844$^{d}$ | |

*Values are Mean ± SD of six individual observations; Values are Statistically Significant at $^{a}P<0.05$ from its respective Controls; Means with different superscripts for each in a column differ significantly at P<0.05*

**Table 3. Performance details of *P. monodon* and *L. vannamei* under different experimental feeding trails at variable stocking densities under polyculture (probiotics + bioflocs added)**

| Parameter | Stocking Ratio 10:10 | | Stocking Ratio 20:10 | | Stocking Ratio 10:20 | |
|---|---|---|---|---|---|---|
| | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** |
| Number of shrimp stocked | 20000 | 20000 | 26500 | 13500 | 13500 | 26500 |
| Percent survival | 85 | 84 | 90 | 94 | 79 | 81 |
| Average Percent Survival | 84.5 | | 92 | | 80 | |
| Final weight (g) | 39.77 ± 0.74[a] | 34.79 ± 0.72[a] | 40.78 ± 0.75[a] | 35.48 ± 0.73[a] | 38.04 ± 0.68[a] | 33.02 ± 0.61[a] |
| Average Final Weight (g) | 37.28[a] | | 38.31[a] | | 35.53[a] | |
| Weight gain (g) | 39.12 ± 0.77[b] | 34.14 ± 0.76[c] | 40.13 ±0.72[b] | 35.19 ± 0.71[c] | 37.39 ± 0.63[b] | 32.37 ± 0.63[c] |
| PDC | 6018[a] | 5252[a] | 6174[a] | 5414[a] | 5752[a] | 4980[a] |
| Average PDC | 5635[a] | | 5794[a] | | 5366[a] | |
| Specific Growth Rates (SGR) | 2.08 | 2.01 | 2.08 | 2.02 | 2.02 | 1.98 |
| Average SGR | 2.05[b] | | 2.05[b] | | 2.00[b] | |
| Daily Growth rates (DGR) (g) | 0.391 ± 0.013[b] | 0.341 ±0.011[c] | 0.401 ± 0.016[b] | 0.352 ± 0.015[c] | 0.374 ±0.014[b] | 0.324 ± 0.013[c] |
| Average DGR (g) | 0.366[b] | | 0.377[c] | | 0.349[d] | |
| Feed Conversion Ratio (FCR) | 2.01[b] | | 1.63[c] | | 1.92[d] | |
| Feed Efficiency (FE) (%) | 50[b] | | 61[c] | | 52[d] | |
| Protein Efficiency Ratio (PER) | 7.49[b] | | 8.45[c] | | 7.74[d] | |
| Productivity (kgs) | 665[b] | 574[c] | 957[b] | 447[c] | 399[b] | 695[c] |
| Average Productivity (kgs) | 620[b] | | 702[c] | | 547[d] | |
| Yield Ratio | 1.16[b] | | 2.14[c] | | 0.574[d] | |
| Total Productivity (kgs) | 1239[b] | | 1404[c] | | 1094[d] | |

*Values are Mean ± SD of six individual observations; Values are statistically significant at [a]P<0.05 from its respective controls; Means with different superscripts for each in a column differ significantly at P<0.05*

**Table 4. Performance details of *P. monodon* and *L. vannamei* under different Experimental feeding trails at variable stocking densities under Polyculture and Monoculture**

| Parameter | Stocking Ratio 20:12 | | Stocking Ratio 20:15 | | Monoculture | |
|---|---|---|---|---|---|---|
| | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** | ***P. monodon*** | ***L. vannamei*** |
| Number of shrimp stocked | 24000 | 16000 | 30000 | 10000 | 40000 | 40000 |
| Percent survival | 85 | 88 | 86 | 88 | 85 | 86 |
| Average Percent Survival | 86.5 | | 87 | | 85.5 | |
| Final weight (g) | 38.14 ± 0.68[a] | 32.11 ± 0.71[a] | 32.72 ± 0.65[a] | 31.14 ± 0.62[a] | 39.77 ± 0.65[a] | 35.02 ± 0.62[a] |
| Average Final Weight (g) | 35.13[a] | | 34.43[a] | | 37.40[a] | |
| Weight gain (g) | 37.49 ± 0.58[a] | 31.46 ± 0.64[a] | 37.07 ± 0.72[a] | 30.49 ± 0.58[a] | 39.12 ± 0.62[a] | 34.37 ± 0.68[a] |
| PDC | 5768[a] | 4840[a] | 5703[a] | 4691[a] | 6018[a] | 5288[a] |
| Average PDC | 5304[a] | | 5197[a] | | 5653[a] | |
| Daily Growth rates (DGR) (g) | 0.375 ±0.011[b] | 0.315 ± 0.010[c] | 0.371 ± 0.013[b] | 0.305 ± 0.012[c] | 0.391 ± 0.012[b] | 0.344 ± 0.011[c] |
| Average DGR (g) | 0.345[b] | | 0.338[b] | | 0.368[c] | |
| Feed Conversion Ratio (FCR) | 1.82[b] | | 1.88[b] | | 2.34[c] | |
| Feed Efficiency (FE) (%) | 55[b] | | 53[b] | | 43[c] | |
| Productivity (kgs) | 765[b] | 443[c] | 956[b] | 268[c] | 665[b] | 591[c] |
| Average Productivity (kgs) | 604[b] | | 612[b] | | 628[c] | |
| Yield Ratio | 1.73[b] | | 3.57[c] | | 1.13[d] | |
| Total Productivity (kgs) | 1208[b] | | 1224[b] | | 1256[d] | |

*Values are Mean ± SD of six individual observations.*
*Values are Statistically Significant at [a]P<0.05 from its respective Controls.*
*Means with different superscripts for each in a column differ significantly at P<0.05.*

**Table 5. Consolidated statement of performance details of *P. monodon* and *L. vannamei* under different experimental feeding trails at variable stocking densities under polyculture operation**

| Treatment | Stocking Ratio10:10 *(P. monodon : L. vannamei)* | Stocking Ratio 20:10 *(P. monodon : L. vannamei)* | Stocking Ratio 10:20 *(P. monodon : L. vannamei)* |
|---|---|---|---|
| | ***Penaeus monodon*** | | |
| Control Group | 28.48 ± 0.65[a] | 30.11 ± 0.58[a] | 27.39 ± 0.61[a] |
| PDC | 4382[d] | 4632[d] | 4214[d] |
| Pribiotic added | 32.24 ± 0.76[b] | 34.11 ± 0.68[b] | 29.77 ± 0.62[b] |
| PDC | 4960[d] | 5248[d] | 4580[d] |
| Probiotic + Biofloc added | 39.12 ± 0.77[c] | 40.13 ± 0.72[c] | 37.39 ± 0.63[c] |
| PDC | 6018[d] | 6174[d] | 5752[d] |
| | ***Litopenaeus vannamei*** | | |
| Control Group | 25.59 ± 0.55[a] | 28.29 ± 0.59[a] | 25.07 ± 0.58[a] |
| PDC | 3937[d] | 4352[d] | 3857[d] |
| Pribiotic added | 29.73 ± 0.56[b] | 32.21 ± 0.69[b] | 28.30 ± 0.62[b] |
| PDC | 4574[d] | 4955[d] | 4354[d] |
| Probiotic + Biofloc added | 34.14 ± 0.77[c] | 35.19 ± 0.61[c] | 32.37 ± 0.63[c] |
| PDC | 5252[d] | 5414[d] | 4980[d] |

*Values are Mean ± SD of six individual observations; Means with different superscripts for each in a row differ significantly at P<0.05; Values are significant at [d]P<0.05 over their respective controls*

## 4. CONCLUSION

The present investigation may be concluded that the results obtained on shrimp polyculture showed that stocking of taxonomically similar species with high density was possible by adjusting the ratios of stocking density between species. The high stocking density, broadcasting of high quality feed, careful manipulation of culture and optimal conditions results in the enhancement of survival, growth and production rates. A stocking ratio of 20:10 with *P. monodon* and *L. vannamei* showed to be optimum inducing highest and best growth potentials thereby production rates were significantly enhanced. From the results obtained in the present study clearly indicates that due to the addition of both Probiotics and Bioflocs, induces the growth potentials significantly in both the species *P. monodon* and *L. vannamei.* Both Probiotics and Bioflocs added into the culture operation are supposed to have different modes of action, but capable of inducing growth potentials and productivity rates. The Probiotics used in the present study indicates growth rates by adopting several modes of action, competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, improvement of water quality, enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemented digestive enzymes. Similarly, due to the addition of carbon sources in the form of Bioflocs into the culture operation facilitates the production of higher levels of plankton, which acts as supplementary materials for and in consumption by the candidate species yields higher productivity rates. The results obtained clearly suggests the use of Probiotics both *B. licheniformis* and *L. rhamnosus* and also Bioflocs in the form of sugarcane molasses for the addition of carbohydrate sources might be playing a promising role and substantially increasing the productivity in the mixed culture operation with two shrimp species *P. monodon* and *L. vannamei.*

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Rosenberry R. World shrimp farming. Summaries of Eastern and Western Hemisphere. Shrimp News International. 1995;1-29.
2. Aghuzbeni SHH, Hajirezaee S, Abbas M, Hossein K, Ghobadi M. A preliminary study on polyculture of western white shrimp *Litopenaeus vannamei* with mullet *Mugil cephalus:* An assessment of water quality, growth parameters, feed intake efficiency and survival. Journal of Applied Animal Research. 2017;45(1):247-251.
3. Lutz CG. Polyculture: Principles, practices, problems and promise. Aquaculture Magazine; 2003.
4. Irz X, Mckenzie V. Profitability and technical efficiency of aquaculture systems in Pampanga, Philippines. Aquacult Econ Manage. 2003;7:195–211.
5. Marcel Martinez-Porchas, Martinez-Cordova LR, Porchas-Cornejo MA, Lopez-Elias JA. Shrimp polyculture: A potentially profitable, sustainable, but uncommon aquacultural practice. Rev Aquacul. 2010; 2(2):73–85.
6. Tian X, Li D, Dong S, Yan X, Qi Z, Liu G, Lu J. An experimental study on closed polyculture of penaeid shrimp with tilapia and constricted tagelus. Aquaculture. 2001;202:57-71.
7. Belton B, Little D. The development of aquaculture in central Thailand: domestic demand versus export-led production. J Agrar Change. 2008;8:123–143.
8. Naresh S. Efficacy of selected Probiotics and Prebiotics in larval rearing and culture of *Penaeid prawns Penaeus monodon* and *Penaeus semisulcatus*. Ph.D Thesis. S.V. University, Tirupati, India; 2016.
9. Avnimelech Y. Carbon / Notrogen ratio as a control in Aquaculture system. Aquaculture. 1999;176:227-235.
10. Walkley A, Black IA. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 1934;37:251-257.
11. APHA. Standard Methods for the Examination of water and waste water (21$^{st}$edition). American Public Health Association, USA; 2005.
12. Hutchinson GE, Mac Arthur RH. A Theoretical model of size distributions among species of Animals. Amer. Natur. 1959;93:145-159.
13. Anil SK, Gunalan B, Jetani KL, Kuldeep Trivedi G, Soundarapandian P. Determine the economic feasibility of the polyculture

system giant tiger shrimp and mullet. Afr J Basic Appl Sci. 2010;2:124–127.

14. Danaher JJ, Tidwell JH, Coyle SD, Dasgupta S, Zimba PV. Effects of two densities of caged mono sex Nile tilapia, *Oreochromis niloticus*, on water quality, phytoplankton populations, and production when poly cultured with *Macrobrachium rosenbergii* in temperate ponds. J World Aquacult Soc. 2007;38:367–382
15. Martinez-Cordova LR, Martinez-Porchas M. Polyculture of Pacific white shrimp *Litopenaeus vannamei,* giant oyster *Crassostrea gigas* and black clam *Chione fluctifraga* in ponds in Sonora, Mexico. Aquaculture. 2006;258:321–326.
16. Muangkeow B, Ikejma K, Powtongsook S, Yi Y. Effects of white shrimp *Litopenaeus vannamei* and Nile Tilapia *Oreochromis niloticus* stocking density on growth, nutrient conversion rate and economic return in integrated closed recirculation system. Aquaculture. 2007; 269:363–376.
17. Wang J, Li D, Dong S, Wang K, Tian X. Experimental studies on polyculture in closed shrimp ponds. Aquaculture. 1998; 163:11–27.
18. Eldani A, Primavera JH. Effect of different stocking combinations on growth, production and survival of milkfish *Chanos chanos* and prawn *Penaeus monodon* in polyculture in brackish water ponds. Aquaculture. 1981;23:59-72.
19. Marichamy R, Rajapackiam S. The culture of milkfish, mullet and prawn in experimental marine fish farm at Tuticorin. In: Proceedings of the symposium on coastal aquaculture. Cochin: Marine Biological Association of India. 1982;6:256–265.
20. Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang JG. Ecological engineering in aquaculture-potential for integrated multitrophic aquaculture (IMTA) in marine offshore systems. Aquaculture. 2009;297:1–9.
21. Srinivasulu Reddy M, Krishna Naik M. Growth performance of *Litopenaeus vannamei* in the presence and absence of probiotics with Bioflocs of different carbon sources. Int. J. fish. Aquatic Studies. 2020;8(5):349-354.
22. Lalitha Kumari CH, Krishna Naik M, Srinivasulu Reddy M. Growth potentials of pacific white shrimp *Litopenaeus vannamei* under different stocking densities with probiotics application. Asian J. Sci. & Tech. 2018;9:7681-7686.
23. Krishna Naik M, Srinivasulu Reddy M. Effect of Biofloc system on growth performance in shrimp *Litopenaeus vannamei* under different C:N ratios with sugarcane molasses. Int. J. Sci. Eng. Res. 2020;11(5):243-262.



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