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ASSESSMENT OF GROWTH CHARACTERISTICS AND THE SURVIVAL RATE OF PACIFIC WHITE SHRIMP *LITOPENAEUS VANNAMEI* (BOONE, 1931), REARED WITH DIFFERENT STOCKING DENSITIES IN THE COASTAL PONDS, SINDH, PAKISTAN

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ABSTRACT

Stocking density is one of the crucial factors affecting the growth and survival of Pacific white shrimp. A suitable density is critical in culturing shrimp, balancing the production as quantity and marketing as quality. Therefore, the present study optimized stocking density for pacific white shrimp reared in the pond ecosystem in Pakistan. The initial weight of postlarvae was (0.003±0.001 grams), and were stocked in 3 stocking densities (Treatment 1- 133.3 PL/m^2, Treatment 2- 300 PL/m^2, and Treatment 3- 466.6PL/m^2) for 114 days. The water quality parameters ranged between (29.3-30.2° C), (5.1-5.5 mg/L), (5.8-7.3), (24.16-16.44 ppt.), (118-120 mg/L), (0.29-0.41 mg/L), (0.97-1.51mg/L), (0.08-0.09 mg/L), (0.02-0.12 mg/L) and (560.66-610 mg/L) for temperature, DO, pH, salinity, alkalinity, phosphate, nitrate, nitrite, ammonium nitrogen and TSS, respectively. Treatment 1 had the highest final weight (24.30±0.90 g), final length (15.8±0.30 cm), average daily gain (0.21g /day), weight gain (24.29 g) and Viscerosomatic index (4.6 g), which was significantly different compared to treatment 2 and treatment 3 ($p<0.05$). SGR did not differ significantly ($p>0.05$) between treatment 1 (7.64 % /day) and treatment 2 (7.63% /day) but was significantly higher than treatment 3 (7.10 %/ day). The Hepatosomatic index and Fulton's condition factor were not significantly varied among the treatments. Treatment 1 had the best FCR (1.11) compared to the other treatments. The survival rate of shrimp significantly varied among these treatments ($p<0.05$) and treatment 1 had the highest survival rate (90.6%), followed by treatment 2 (82.20%) and treatment 3 (75.38%). Moreover, polynomial regression showed that 133.3 PL/m^2 to 240 PL/m^2 is an excellent stocking density for *L. vannamei.* Furthermore, the stoking density from 240 PL/m^2 to 460 PL/m^2 decreased the growth and survival rate. The present study concluded that the stocking density of *L. vannamei* at 250-300 PL/m^2) can achieve the best growth and survival rate.

Keywords: *Litopenaeus vannamei*, Stocking density, Growth performance, Survival

Abbreviation: ADWG: Average daily weight gain; FCR: Feed conversion ratio; WG: Weight gain; SGR: Specific growth rate; TL: total length; CF: Condition factor; HSI: Hepatosomatic index; VSI: Viscerasomatic index; SR: Survival rate

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INTRODUCTION

The demand for animal protein has increased with the increasing population and the rising global warming, which is declining the wild stocks in some countries, leading to a crisis of food. Hence, aquaculture plays a significant role in food and nutrition security and is a source of people's livelihoods (Hassan *et al.,* 2021a,b; Abidin *et al.,* 2022). Also, aquaculture is the world's fastest-growing food production system at an annual rate of 8% and contributes 44.1% of the world fish supply of 167.2 million tons (FAO, 2016; Hassan *et al.,* 2021c). Shrimp farming has become a significant factor in world shrimp markets over the past five to six years and contributed to 6.9% of the total output (FAO-FIGS, 2018).

Furthermore, shrimp remained a high-value product with a vast international market and is used in the fish feed industry. White leg shrimp or king prawn *Litopenaeus vannamei* is one of the important aquaculture species worldwide (Alcivar *et al*., 2007). This species has demand in home and export markets (Zulkarnain *et al.,* 2020). Pacific white shrimp has many advantages, such as it lives in a wide range of salinity 0.5 to 45 ppt, a rapid growth of 3.5 g/week and a feed conversion rate between 1.2 and 1.6 (Jaffer *et al.,* 2020).

Stocking density is one of the essential factors considered the key to the success of the aquaculture sector due to its effects on water quality (Li and Wang, 2006), growth performance, survival, behaviour, health, feed utilization and disease control (Lin *et al.,* 2004). Stocking density considers the best means for exploiting the area unit and increasing production (Tantu *et al.,* 2020). Many studies have shown an inverse relationship between stocking density and growth (Castro *et al.,* 2019). The optimum stocking density of shrimp has been extensively studied for different shrimps/aquaculture, such as *Penaus semisulcatus* (Zaki *et al.,* 2004), *Fenneropenaeus merguiensis* (Anand *et al.,* 2014), *Farfantepenaeus paulensis* (Wasielesky *et al.,* 2001), *P. indicus* (Sivanandavel *et al.,* 2010) and *P. monodon* (Duy *et al.,* 2012). The stocking density of culture white shrimp varies according to different production systems and cultivation ponds. Despite many studies conducted to determine the optimum stocking density of white shrimp, the positive effect of stocking density still needs clarification and little information about the stocking density of *L. vannamei* is available in Pakistan. The present study investigated the effects of stocking densities on the growth performance and the survival of the Pacific white shrimp *L. vannamei* reared in the pond under the intensive aquaculture system.

MATERIALS AND METHODS

Experimental design: This study was carried out in the pond's ecosystem in Sindh, Pakistan (N: 24.546767, E: 67.46097). Postlarvae of *L. vannamei* were imported from Thailand SY AQUA (Thailand) commercial hatchery; they were verified negatively for Taura Syndrome Virus (TSV) and White Spot Syndrome Virus (WSSV) by Polymerase Chain Reaction (PCR Assay) before packing. Postlarvae were put in oxygenated double-coated polythene bags to reduce stress during transportation. Ice packages are also used between the internal and external coverings of polythene bags to sustain optimal temperature during shrimp seed transportation. The postlarvae were brought to the farm site early in the morning. Water was supplied to the postlarvae bag gradually to maintain salinity and pH. The seeds were stocked into the ponds (1.5-hectare area) slowly at a depth of 4.02±1.62 ft. The acclimatization period (two weeks) was performed before the experimental period. The total number of larvae was 1350000 with an average initial weight of 0.003±0.001 g was randomly distributed into ponds at three stocking densities (Treatment 1- 133.3 PL/m^2, Treatment 2- 300 PL/m^2, and Treatment 3- 466.6 PL/m^2). Each treatment was triplicated. A diet containing 35% protein was used in this study (Table 1). Postlarvae fed four times daily at 10% biomass as the feeding rate. The feeding rate is adjusted according to shrimp biomass every week. Circular trays were used for feeding. Four Paddles wheel aerators in each pond were used to sustain the level of dissolved oxygen (DO) in the experimental ponds. The water exchange rate was 40% of the total water volume twice per week. The length of the experimental period was 114 days. A cast net was used for sampling to track the shrimp's health and growth every week to quantify the various parameters.

Table 1: Amount of different feed ingredients (g/100 g) used in the experimental diet and the proximate chemical composition of the diet.

Ingredients	Experimental diet (g/100 g)
Squid liver meal	5.0
Fish meal	28.0
Gelatin	2.50
Casein	8.0
Wheat flour	26.5
Starch	18.0
Choline chloride	1.0
Lecithin	1.0
Soybean meal	7.0
Fish oil	1.0
Vitamin/mineral premix*	2.0
Chemical composition	**(%, based on dry matter weight)**
Dry matter	88.4
Crude protein	35.8
Crude lipid	8.18
Crude ash	5.15
Total carbohydrate**	50.87
Gross energy KJ/g***	20.62

Mineral/ vitamin premix (g kg^{-1} of mixture): retinol, 3.0; cholecalciferol, 1.0; ascorbic acid, 20.0; tocopherol, 20.0; riboflavin, 6.0; menadione, 2.0; cobalamin, 6.0; thiamine, 4.0; pyridoxine, 5.0 inositol, 54.0; biotin, 0.2; niacin amide, 40.0; folic acid, 2.0; Ferric citrate, 10.0; pantothenic acid, Zn, 30.0; 12.0; I, 1.0; Cu, 1.0; Se, 0.01; Co, 10.0; Mn, 2.0; K, 6.0;

Ponds preparation and fertilization: To restore ponds, 3-6 cm of bottom soil was removed, which helped avoid toxins and heavy metals. Ponds were sun-dried for a week and then levelled. Inorganic nitrogen and phosphorous fertilizers were provided in shrimp ponds to stimulate phytoplankton and increase the zooplankton community. Dolomite was used to help in plankton growth. Liming shrimp ponds was carried out to nullify the acidity and improve the overall alkalinity and total hardness of the water.

Water quality parameters: Physicochemical parameters like temperature, salinity, pH, and dissolved oxygen (DO), were measured daily by a Celsius glass thermometer, Handheld Refractometer, A mobile digital DO-meter (Model: HI9146), and Digital pH meter, respectively, while alkalinity, ammonia, nitrite, and nitrate by chemical methods according to (APHA, 1995).

Calculation of growth parameters: An average daily weight gain (ADWG) Feed conversion ratio (FCR), weight gain (WG), specific growth rate (SGR), total length (TL), condition factor (CF), Hepatosomatic index (HSI), Viscerasomatic index (VSI), and survival rate, these indices were calculated by Szkudlarek & Zakes (2007), and Hassan *et al.* (2020)

ADWG= {(Final weight-Initial weight)/Days}

WG=Final weight-initial weight

SGR = (ln (final weight in grams) - ln (initial weight in grams) x 100) / t (in Days)

FCR=Food given (g)/Weight gain (g)

SR (%) = (No. of fish survived/ No. of fish released) ×100

Condition factor = (Weight/Length3) ×100

VSI = {Wet weight of visceral organs and associated fat tissue (g)/wet body weight} ×100

HSI=weight of the liver (g)/empty shrimp weight (g) ×100.

Chemical analysis of feeds: The chemical analysis of feed was conducted according to (AOAC, 2000), and Gross energy (GE) was estimated for formulated diets. The factors 23.62, 39.5 and 17.56 KJ/g for CP, EE and carbohydrates were used (NRC, 1993).

Statistical analysis: All data were checked for normality and homogeneity of variance. Data were presented as a mean± SEM. A repeated measure ANOVA was performed to see the effects of different stocking densities on average daily weight gain, total weight gain, specific growth rate and feed conversion ratio, different health indices, and shrimp survival rate. Polynomial regression was done using SPSS software (version 17.0). The significance difference level of $p<0.05$ was set for all analyses.

RESULTS AND DISCUSSION

Physicochemical parameters: Water quality parameters like temperature, dissolved oxygen, pH, salinity, alkalinity, phosphate, nitrate, nitrite, total ammonium nitrogen, and total suspended solids were determined each week and presented in Table 2. Statistical analysis confirmed significant variations among the treatments in dissolved oxygen, phosphate, pH, nitrate, total ammonium nitrogen, and total suspended solids ($p<0.05$). Treatment 3 had the highest phosphate concentration (0.41 mg/L), nitrate (1.51 mg/L), ammonia (0.12 mg/L) and TSS (610 mg/L), respectively followed by Treatment 2 and Treatment 1. The water quality parameters ranged between (29.3-30.2° C), (5.1-5.5 mg/L), (5.8-7.3), (24.16-24.44 ppt.), (118-120 mg/L), (0.29-0.41 mg/L), (0.97-1.51mg/L), (0.08-0.09 mg/L), (0.02-0.12 mg/L) and (560.66-610 mg/L) for temperature, DO, pH, salinity, alkalinity, phosphate, nitrate, nitrite, ammonium nitrogen and TSS, respectively.

Table 2: Water quality parameters in ponds during the experimental trial. Data shows as Mean of three replicates ± Standard deviation and different superscript letters in the same row denoted significant variance (p<0.05).

Parameters	Treatment 1	Treatment 2	Treatment 3
Temperature (°C)	30.2±3.10	30.1±2.80	29.3±3.20
Dissolved oxygen (mg/l)	$5.5±1.20^{a}$	$5.5±2.11^{a}$	$5.1±1.20^{ab}$
pH	$7.2±1.06^{a}$	$7.3±2.12^{a}$	$5.80±1.20^{b}$
Salinity	24.44±16.22	24.23±16.42	24.16±16.78
Alkalinity (mg/l)	118±3.12	120±1.11	120±2.12
Phosphate (mg/l)	$0.29±0.07^{b}$	$0.38±0.1^{a}$	$0.41±0.2^{a}$
Nitrate (mg/l)	$0.97±0.07^{c}$	$1.19±0.09^{b}$	$1.51±0.21^{a}$
Nitrite (mg/l)	0.08±0.01	0.08±0.20	0.09±0.02
Total ammonium nitrogen (mg/l)	$0.02±0.0.01^{c}$	$0.04±0.02^{b}$	$0.12±0.06^{a}$
Total suspended solids (mg/l)	$560.66±8.88^{c}$	$580.20±6.78^{b}$	$610±35.44^{a}$

The physicochemical parameters of water play a crucial role in aquaculture systems. It can directly affect the health status, growth, feed utilization, and survival of aquatic animals (Xu *et al.,* 2018; Kumari *et al.,* 2018; Maicá *et al.,* 2014; Leal *et al.,* 2019). The maintenance of water quality is essential for the optimum growth performance and survival of shrimp. Although some of the water quality parameters in the present study indicated significant variations, all mean values fell between the suitable range for the optimum growth and health of Pacific white shrimp (Yua *et al.,* 2020). The effect of water quality on shrimp growth and the survival rate has been extensively studied; Mena-Herrera *et al.* (2006) showed that shrimp rearing under low density and suited temperature provided the best growth performance. The water quality parameters are closed related to the stocking density. The high stoking rate is accompanied by reducing the pH and decreasing DO (Gaber *et al.,* 2012). In a previous study, the best growth and survival rate of *L vannamei* were achieved at 24-32 °C, pH 7.6-8.6 and 10 to 35 PSU salinity (Wang *et al.,* 2004; Gunalan *et al.,* 2010).

Growth performance: The growth parameters indicated that different stocking densities significantly affected white shrimp postlarval growth performance (Table 3). The highest growth performance was observed in treatment 1. Treatment 1 had the highest final weight (24.30±0.90 g), final length (15.8±0.30 cm), average daily gain (0.21g /day), weight gain (24.29 g) and Viscerosomatic index (4.6 g), which was significantly different compared to treatment 2 and treatment 3 ($p<0.05$). SGR did not differ significantly ($p>0.05$) between treatment 1 (7.64 % /day) and treatment 2 (7.63% /day) but was significantly higher than treatment 3 (7.10 %/ day). The Hepatosomatic index and Fulton's condition factor were not significantly varied among the treatments.

Table 3. The growth performance of Pacific white shrimp reared at various stocking densities for 114 days. Values are shown as the mean ± standard deviation, and different superscript letters in the same row denoted significant variance (p<0.05).

Parameters	Stocking density		
	Treatment 1	Treatment 2	Treatment 3
	133.3 PL /m^2	300 PL/ m^2	466.6 PL /m^2
Initial body weight (g)	0.004±0.001	0.003±0.001	0.004±0.002
Final body weight (g)	24.30±0.90^a	18.10±0.64^b	13.11±0.66^c
Final body length (cm)	15.8±0.30^a	14.24±0.21^b	11.8±0.40^c
Specific growth rate (%d^{-1})	7.64±0.03^a	7.63±0.05^a	7.10±0.04^c
Feed conversion ratio	1.11±0.24^b	1.40±0.02^a	1.50±0.01^a
Average daily weight gain (g/day)	0.21±0.007^a	0.15±0.005^b	0.11±0.005^c
Weight gain (g)	24.29±0.007^a	18.09±005^b	13.10±0.005^c
Viscerosomatic index	4.60±0.3^a	4.12±0.2^b	3.60±0.2^c
Hepatosomatic index	3.32±0.2	3.18±0.1	2.99±0.1
Condition factor	0.61±0.00	0.62±0.00	0.79±0.00
Survival rate (%)	90.60±2.18^a	82.20±3.18^b	75±3.88^c

Shrimp farms are profitable in the aquaculture industry (Tacon *et al.,* 2002; Hassan *et al.,* 2021). Determining the optimum stocking rates in each production system leads to more economic returns. Mena-Herrera *et al.* (2006) stated that the extensive system in shrimp farms has a stocking rate of 1 to 3 shrimp/m^2, semi-intensive from 10 to 50 shrimp/m^2 and intensive are 50 to 160 shrimp/m^2. The present study tested three different stocking rates (T1) 133.3 PL/m^2, (T2) 300 PL/m^2 and (T3) 466.6/m^2. Current results found the high-density effects on growth performance, survival, health condition, feed utilization and production. The growth indicated that the growth performance of *L. vannamei* improved with low stocking density. Our finding was in stark agreement with many previous works (Forster and Beard, 1974), which raised nine shrimp species under two stocking rates, and they observed an adverse effect of density on performance. The growth rate of *L. vannamei* was affected by increasing the stocking rates Williams *et al.* (1996). Moss and Moss (2004) mentioned a negative curvilinear relationship between shrimp growth and increasing density. In the same trend, a negative relationship between the stocking rates and growth performance of *Macrobrachium* was found in different culture systems (Marques *et al.,* 2000). A previous study on *Penaeus mondon* showed a significantly negative correlation between individual growth and stocking density (Chakraborty *et al.,* 1997). High stocking density leads to a reduction in rearing area and increasing competition for feed. Also, the high density leads to greater dominance and aggressive behaviour of the large shrimp on the small shrimp in the same pond. The amount of feed present in the pond area bigger with high stocking density increase the levels of turbidity and metabolic wastes. The stress from a high stocking density results in immune depression in shrimp (Huang & Chiu, 1997; Ahmed *et al.,* 2000; Moraes-Valenti *et al.,* 2010; Gaber *et al.,* 2012; Arnold *et al.,* 2006). On the contrary, Wyban *et al.* (1988) differed from our findings and argued that *L. vannamei*'s growth in ponds was not affected by stocking density.

Feed conversion ratio and survival: In this study, FCR was significantly affected by the stocking density. The best feed conversion ratio for shrimp postlarvae was obtained with a low stocking density. Treatment 1 had the best FCR (1.11) compared to the other treatments. No significant influence of increasing the density from 300 to 466.6 PL/m^2 was observed with treatment 2 (1.40) and in treatment 3 (1.50) ($p>0.05$). The survival rate of shrimp significantly varied among these treatments ($p<0.05$) and treatment 1 had the highest survival rate (90.6%), followed by treatment 2 (82.20%) and treatment 3 (75.38%).

The feed conversion ratio had a negative effect with high stocking density. In contrast, significantly better FCR was observed in treatments 1 and 2 than in treatment 3. The high stocking density led to reduced feed intake because of social dominance and the amount of energy consumed due to competing with the individuals during feeding time. A similar result was reported by Sugathan *et al.* (2014). Moreover, a low stocking rate recorded the best FCR and survival rate of tiger shrimp (Gaber *et al.,* 2012). Besides this, the survival rate adversely relates to high stocking density in *L. vannamei* (Munoz *et al.,* 2019; Parvathi and Padmavathi, 2018; Shrivastava *et al.,* 2017; Washim *et al.,* 2016). Kumar & Krishna (2015) evaluated five different stocking rates on *L. vannamei* (20, 30, 40, 50 and 60 PL/m^2) and noted a decrease in survival with increasing stocking density. Similar results were found with other shrimp species such as *Penaeus monodon* (Sugathan *et al.,* 2014; Zaki *et al.,* 2004; Krishna *et al.,* 2015), *Fenneropenaeus merguiensis* (Anand *et al.,* 2014). Reduced survival with high stocking density is possibly due to cannibalism in crustaceans through the moulting process (Andi *et al.,* 2013).

The optimum stocking density achieves the best FCR, SGR, and survival rate. It could be said that the optimum stocking density is the maximum density that not negatively affects SGR, FCR and survival rate. The broken line regression between SGR and stocking density showed that 250 PL/m^2 is the optimum stocking density for *L. vannamei* under the intensive system in the coastal pond of Pakistan (Fig. 1). Generally, stoking density is inversely proportional to shrimp growth, and the optimum stocking rate depends on managing water quality, age and species of shrimp and rearing season. Mena-Herrera *et al.* (2006) found that the effects of stocking rates on shrimp growth dramatically differed between the seasons. They argued that the growth rate of shrimp did not change among different stocking densities in the winter, but in the summer, the growth rate varied with the same stocking density. Stocking density was positively related to the total production per area unit and negatively correlated with a growth rate per animal unit (Gaber *et al.,* 2012).

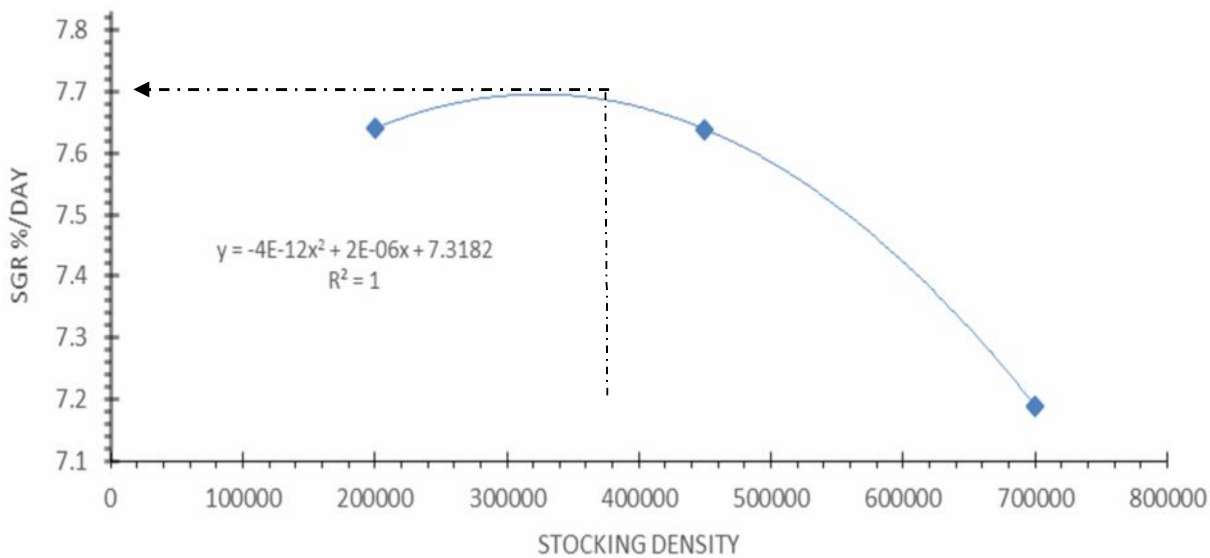

Figure 1. The second-order polynomial regression between stocking density and specific growth rate (SGR %/d) showed that 380000 individuals of *L. vannamei* are the suitable range for shrimp culture under 1.5 hectors pond (250 PL/m^2).

Conclusion: The rising of commercial aquaculture slightly depends on boosting growth performance and survival rate for aquatic animals in the aquaculture environment. The growth and survival of shrimp were significantly related to stocking density. The present study showed that increasing stocking density leads to decreased growth, survival, and the trouble of management, and reduced stocking density leads to increased weight gain, final weight, specific growth rate, survival rate, and improvement FCR. It can be concluded that the optimum stocking density for the production of Pacific white shrimp reared under an intensive system in the coastal pond is ranged between 250-300 PL/m^2. The findings of the present study have practical implications and could be considered a major step for optimizing stocking density and the development of shrimp culture technology in Pakistan.

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