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Stocking density, Survival rate and growth performance of *Litopenaeus vannamei* (Boone, 1931) in different cultured shrimp ponds from Vetapalem, Prakasam District, Andhra Pradesh, India

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Abstract
The present study is the report on the culture of *L. vannamei* in the brackish water shrimp ponds in vetapalem, Chirala, Prakasam district, Andhra Pradesh, India. The study was conducted in four culture ponds at different stocking densities. Crab fencing and bird netting was done before pumping water to prevent the auto entrants. The average pH reading for the AM was 7.8 to 8.2, while a pH of 8.3 to 8.9 was recorded in the PM. After 110th days of pond culture, the mean average weights of the shrimp at harvest were 19.5, 19.8, 19, and 19.8; survivals were 71, 66, 74, and 73 %; FCR was 1.7, 1.6, 1.7, and 1.7. The average production was 7000, 8000, 7200, and 7300 kg/ha for P1, P2, P3, and P4, respectively. Two way ANOVA was attempted between stocking density and Average body weight (ABW). The calculated p value was 0.0015. Its highly significant. So ABW increases with less stocking density and vice versa. The present investigation, it was concluded that *L. vannamei* culture is successful in brackish water environment and the growth is directly related to stocking density.

Keywords: *Litopenaeus vannamei*, growth performance, stocking densities, water quality, statistical analysis

Introduction
The coastal aquaculture shrimp production to over 5 lakh tonnes consistently in the last two years has helped boost the seafood exports from India, according to Marine Products Export Development Authority (MPEDA). 14 MAY 2017. *Litopenaeus vannamei* is the most important penaeid shrimp species farmed earth (Surya *et al*., 2016). During the last few years, white spot disease (WSD) has spread worldwide and caused large scale mortalities and severe damage to shrimp culture, particularly in Asia leading to massive economic losses (Lightner, 1996; Flegel, 1997) [11, 7]. Due to continues outbreak of WSSV in of *P. monodon* culture leads to shattering of shrimp culture in India. So the farmers are seriously looking for alternative species for culture. At right time the Coastal Aquaculture Authority of India (CAA) introduced a new species *(Litopenaeus vannamei*) in India. At the same time CAA is very keen in the bio security and approval for culture of *L.vannamei*. Shrimp farming is one of the majority gainful and fast growing sector in a aquaculture industry (Tacon, 2002) [24]. Unfortunately, this manufacturing has suffered drastic collapses from decrease growth and survival as an increase in stocking density.

The shrimp has been introduced and farmed in Asia since the mid 1990s, with production in Mainland China being particularly significant. However, beginning in 1996, *L. vannamei* was introduced into Asia on a commercial scale. Total production of *L. vannamei* in Asia was approximately 316000 mt in 2002. (Balakrishnan *et al*., 2011) [1]. In some countries, *L. vannamei* culture has been promoted by some private sector suppliers as being tolerant or resistant to WSSV, leading to introductions based on a mistaken belief that they are safe. It is now evident that *L. vannamei* is farmed and established in several countries in East, Southeast and South Asia and is playing a major significant role in shrimp aquaculture production. Several authors described concerning the growth in shrimp culture systems based on stocking density (Cailout *et al*., 1976; Sedgwick 1979; Maguire and Leedow 1983). There is very limited research works were done on the culture and growth performance of *L. vannamei* with different stocking densities in brackish water ponds in India. So the present study was endeavoured to evaluate the survival, growth, and FCR of *L. vannamei* culture in the brackish water with different stocking densities.

Materials and Methods
The present study was undertaken at shrimp ponds ($15'78^0$ N and $80'32^0$ E) in Vetapalem, Chirala, Prakasam district, Andhra Pradesh, India. The study was conducted in four shrimp culture ponds. Three ponds (1, 3 and 4) were 1ha and pond 2 was 1.5 ha in area. Besides the above there was a reservoir pond in the size of 3 ha, a sedimentation pond and a chlorination pond are in the size of 0.6 ha. Water recirculation method followed to avoid cross contamination during the culture period. All the experimental ponds were 1.2 – 1.5m deep. The soil type was sandy clay. Ponds were initially prepared by drying, tilting (to remove the pests and predators and oxidize bottom soil) and liming to correct the pH of the soil. Inorganic fertilizers such as urea and triple superphosphate were applied to enrich the natural food organisms in the water. Crab fencing and bird netting was done before pumping water to prevent the auto entrants. The filter bags were checked properly, which was fitted in the inlet and outlet pipe, then the pumping was done to the entire ponds. After filling water kept stand one day without any disturbance for sedimentation. Subsequently the water was chlorinated (60 ppm/ha) after that excess chlorine was neutralized by dechlorination process

which took 72 hours. After dechlorination, the water enriched with probiotic for the good beneficial bacterial environment. The algal bloom was noticed slowly in the ponds.

The *L. vannamei* seeds (post larval stage 14, that had been acclimated to a salinity level of 17 ppt and confirmed negative for the white spot syndrome virus(WSSV) and Taura syndrome virus (TSV) by the polymerase chain reaction(PCR assay), were purchased from CP Aquaculture India Private Ltd, hatchery Gudur. The seeds were transported in oxygenated double-layered polythene bags with crushed ice packs between inner and outer covers of the bag to maintain optimum temperature in turn to keep less stress to the shrimps and the entire set up was packed in a carton. The seeds were brought to the farm site and bags were kept in the pond water for some time to adjust the temperature. Then the pond water was added slowly into the seed bag to adjust the salinity and pH. Subsequently the seeds were released slowly in to the ponds. The stocking densities were 50/ m², 40/m², 51/m² and 56/m² for ponds 1, 2, 3 and 4 respectively.

Blanca feed pellets (CP Aquaculture India Pvt Ltd) were fed to the stocked post larvae for four times daily at 7am, 10am, 3pm and 9pm respectively. No water exchange was done for the entire culture period. But some water from the reservoir was added at regular intervals to compensate water loss due to evaporation or soil seepage. During harvest all the water from culture ponds drained to sedimentation pond and ultimately reached to reservoir pond. At any account of time the pond water was not pumped out side of the farm as a bio secure measures. From the 60th day of culture (DOC) onwards cast net (sampling) was used every seven days for monitoring shrimp health and growth.

The water level was measured by using a standard scale with cm marking. The water quality parameters like salinity, pH, temperature (^{o}C) measured thermometer, dissolved oxygen and light transparency were measured by using hand refractometer (Erma, Japan) the pH of the water by using a calibrate pH pen (pH esp.-3 model) thermometer, and dissolved oxygen meter and secchi disc, respectively. Aeration was given to the entire culture period for all ponds. Totally 16 hp aerator was fixed for each culture pond. The aerators were placed in such a way that it could dissolve maximum dissolved oxygen (DO) into the pond water and makes the culture environment friendly. Feed conversion ratio (FCR) and average daily growth (ADG) were calculated by the given formula below

Growth performance

The growth performance of the shrimp was measured based on mean individual weight at harvest, total weight gain growth rate, feed conversion ratio (FCR) and survival rate total weight gain, growth rate, FCR and survival rate was calculated.

FCR = Total weight of the harvested shrimps / total feed used
ADG = Total weight gained by the shrimps / Total days of culture

Statistical Analysis

Two way ANOVA analysis was applied to know the statistical significance between growth and stocking densities of the shrimp. Data were expressed in the mean ± standard error.

Results

Water quality analyses for the culture ponds are summarized in Tables 1&2. Pond water pH, temperature and DO readings are recorded in early mornings (AM) and late evenings (PM). For the four culture ponds an overall, average fluctuation of pH reading was between 7.8 and 8.2 in the early morning, while fluctuation of pH value was between 8.3 and 8.9 in the evening. DO values fluctuated varied between 4.2 mg/l and 3 mg/l in the morning and between 4.5 mg/l and 10 mg/l in the evening (Table 2). In general, early morning readings became lower as the cycle progressed and the standing crop increased. Average AM and PM pond temperatures were 23 to 30° C, respectively (Table 1). In general, the temperature trend through the production cycle started with temperatures around 27.5 °C, dropped to 22 °C because of a cold front during the third and fourth week, and then increased to a range of 28–29°C. During the culture period the maximum salinity was recorded 25ppt and minimum salinity was recorded 18ppt in all the ponds.

Table 1: Average water quality parameters

Parameters	Range
Salinity (ppt)	18-25
Temperature ˚C	23-30
pH AM	7.8-8.2
pH PM	8.3-8.9

Weekly averages of shrimp weights are presented in Table.3. After 110th days of pond culture, the mean average weights of the shrimp at harvest were 19.5g, 19.8g, 19g, and 19.8g (Table 4); survivals were 71, 66, 74, and 73 %; FCR was 1.7, 1.6, 1.7, and 1.7. The average production was 7000, 8000, 7200, and 7300 kg/ha for P1, P2, P3, and P4, respectively (Table 4).

Table 2: Average dissolve oxygen concentration

Days of culture (DOC)	1	10	20	30	40	50	60	70	80	90	100	110	120
Morning (mg/l)	3	3.5	4	4.2	4	4.2	4.5	4	3.9	4	3.9	3.5	3.8
Evening (mg/l)	5.1	6.5	6	7	7	8.5	7	6.8	7	8	9	10	8.7

Table 3: Weekly growth performances (g)

Ponds	Days Of Culture (DOC)								
	60	67	74	81	88	95	102	109	116
P1	8	9.2	10	11.5	12.8	14.5	16.9	17.6	19.5
P2	8.1	9.5	11	12.2	13.5	15	16.5	17.5	19.8
P3	8.2	9.1	10.5	11.9	13	15	16.3	17.5	19
P4	8	9	10.4	11.8	13.7	15.2	16.5	18	19.8

Table 4: Pond performance details

Details	Pond 1	Pond 2	Pond 3	Pond 4
Area (Ha)	1	1.5	1	1
Initial Stocking (Numbers)	500000	600000	500000	500000
Density (Numbers)	50	40	51	56
Stocking Date	5-Sep-17	5-Sep-17	6-Sep-17	6-Sep-17
Harvest Date	12-Jan-18	14-Jan -18	15-Jan-18	16-Jan-2018
Culture Period	116	118	119	120
Harvest Size (g)	19.5	19.8	19	19.8
Count (Numbers/Kg)	51	50	52	50
Shrimp Harvest (Kg)	7000	50	52	50
Survival Percentage	71	66	74	73
Total Feed Used (Kg)	12000	13000	12500	13000
FCR	1.7	1.6	1.7	1.7
ADG	0.16	0.16	0.15	0.16

Table 5: Average cost analysis

Area (ha)	**4.5**
Density(Numbers/m^2)	49
Harvest Size	19.5
Count(numbers/kg)	50
DOC	118
Survival (%)	71
FCR	5.6
Production (kg)	29500
Total feed (kg)	50500
Seed cost /kg shrimp	18.5
Feed cost /kg shrimp	62.5
Pond preparation cost /kg	1
Water treatment cost /kg	3
Feed probiotic cost /kg	2.5
Water probiotic cost /kg	1.5
Bottom probiotic cost /kg	2
Carbon source cost / kg	3.2
Minerals cost / kg	3.5
Chemicals cost /kg	3.4
Feed supplement cost /kg	2.5
Diesel cost /kg	6
Electricity cost/ kg	16.25
Labour cost/ kg	10.90
Farm lease cost/ kg	27.27
Maintenance & repair/ kg	10.90
Other expenses /kg	14.5
Total production cost (Rs)/ kg of shrimp	189.52
Material price (Rs)	250
Profit /kg	60.48
Total profit (Rs)	590525

For each pond cost analysis was worked out. Production cost for 1kg shrimp (19.5 gram, 50 counts) was calculated as Rs 189.52. The feed cost was Rs 62.5/kg, followed by seed cost Rs 18.5/kg. The overall production was 29,500 metric tons. Totally 50.500 metric ton feed was used. The average FCR was 5.6, average ABW was 19.5g and average density was 50 numbers/m². Profit /kg shrimp was Rs 60.48 and overall total profit was Rs 590525 (Table 5).

Two way ANOVA was attempted between stocking density and Average body weight (ABW). The calculated p value was 0.0015. Its shows the relationship between stocking density and average body weight is highly significant So ABW increases with less stocking density and vice versa.

Discussion

The present study is the report on the culture of *L. vannamei* in the brackish water shrimp farm in Vetapalem, Chirala, Prakasam district, Andhra Pradesh, India. This study shows that stocking density affects growth of *L. vannamei*. Several authors have reported on the growth and survival of *L. vannamei* in different salinities and densities (Wyban, *et al*., 1988, Samocha, *et al*., 1993, Samocha, *et al.*, 1999, and Emberson CR, *et al.,* 2008) [27, 17, 6, 13, 19]. The maintenance of good water quality is essential for optimum growth and survival of shrimp. Good water quality characterized by adequate dissolved oxygen, temperature, pH and salinity. Excess feed, faecal matter and metabolites will exert tremendous influence on the water quality of the shrimp ponds (Soundarapandian and Gunalan, 2008) [21]. In the present study the salinity was maintained 18 – 25 ppt in all ponds. However, the white leg shrimp, *L. vannamei* is widely cultured in Central and South America (Balakrishnan, *et al*., 2011) [1, 2]. and tolerates the salinities of 2-45 ppt (Parker, *et al.,* 1974 [14], Samocha, *et al.*, 1998) [18]. Several authors have reported good growth and survival of *L. vannamei* in brackish water of 1.7-2.3 ppt (Bray WA, et al., 1994, Bray, *et al*., 1999, Moya *et al*., Samocha et al., 1999 and Emberson, *et al.,* 1999) [4, 3, 3, 13, 19, 6, 13, 19, 6]. (Karthikean, 1994, Gunalan, *et al.*, 2010) [9]. recommended a salinity range of 10 – 35 ppt was ideal for shrimp culture. (Samocha *et al*., 2004 Sowers, and Tomasso, 2006) [20] [22]. reported that growth is higher in low saline (2

ppt) water than in sea water. In the present study pH value was ranging between 7.8– 8.2 in the morning and 8.3-8.9 in the evening. The pH of pond water is influenced by many factors, including pH of source water, acidity of bottom soil and shrimp culture inputs and biological activity. (Wang *et al*., 2004) recommended the favorable pH range of 7.6-8.6 for *L. vannamei*. The concentrations of DO in all ponds are ranged from 3.5- 4.2 mg/l in the morning and 4.5-10 mg/l in the evening during the culture period. The values of water quality parameters reveal that all these are in the acceptable range for survival and growth for *L. vannamei* (Van Wyk, 1999, Wickens, 1976) [25, 26].

The growth of the shrimps depends on the quality of feed. In the present study CP feed was used for all the ponds and the amount was followed as per feed chat. The maximum feed was used in pond P2 followed by P4, P1 and P5. In the present study the average FCR was 5.6 for all ponds. Similar results were recorded (Paul Raj, 1999, Ramakrishna, 2000 and Soundarapandian and Gunalan, 2008) [15, 16, 21]. even though the stocking densities was quiet high could able to achieve the better FCR in all the ponds because of quality of the feed, feed management, water quality, pond bottom management and other effective farm management. Weekly sampling is very important to know the shrimp health, growth and survival. In the present study first sampling was carried out in all ponds at the 55th DOC of the culture. During sampling the growth of the shrimps varied depends on the density. At the time of harvest in P1pond the shrimps harvested at the size of 19.5 grams, inP2 pond 19.8 grams, in P3 pond 19 grams and in P4 pond 19.8 grams. The higher survival (74%) was recorded in the pond P3 and lowest survival (66%) was recorded in pond P2. (Bray, *et al*., 1994) [4]. was observed similar finding in their research. Shrimp survival was quite well considering the dimension of the pond and the sanitary risks of outdoor-reared shrimp (Green, *et al.*, 1997; Martinez- Cordova, 1998) [8, 12]. The appreciable feed conversion ratio indicates good rearing procedures combined with a suitable environment and a good shrimp biological responsiveness. From the present investigation it was concluded that *L. vannamei* culture is successful in brackish water environment and the growth is directly related to stocking density.

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