Effect of Stocking Density on the Survival Rate and Growth Performance in *Penaeus monodon*

C. Shakir[1], A.P. Lipton[2], A. Manilal[3], S. Sugathan[4,*] and J. Selvin[5]

[1]*Department of Microbiology, Bharathidasan University, Trichy, India*

[2]*Central Marine Fisheries Research Institute, Vizhinjam 695 521, Thiruvananthapuram, India*

[3]*College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia*

[4]*Department of Botany and Biotechnology, Sree Narayana College, Kollam, Kerala, India*

[5]*Microbiology Programme, Pondicherry University, Puducherry, Tamil Nadu, India*

Abstract: In this study, effect of stocking density and water exchange rate on the survival, growth and apparent feed conversion ratio of *Penaeus monodon* were evaluated. For that, shrimps were cultured at two different stocking densities such as six and twelve post larvae m^{-3} for a period of four months. The experimental ponds were harvested after four months of culture. The results revealed that survival rate of shrimps cultured in low density ponds (LD) was higher (68.4%) whereas a lower rate (51.7%) was observed at high density ponds (HD). In the case of average body weight and length, the shrimps reared at LD experimental ponds were 27.8 g and 18.5 cm respectively. In addition, the average FCR value of shrimps reared at LD ponds was higher (1.8) when compared to the shrimps cultured in HD ponds (2.3). Therefore, stocking density of 6 PL/m^3 is recommend for culturing *P. monodon* in Kollam coast of India under these experimental conditions.

Keywords: *Penaeus monodon*, shrimp farming, stocking density, survival rate.

INTRODUCTION

Shrimp farming is an aquaculture business industry in many developing countries for earning foreign exchange and providing employment to the coastal people [1]. The demand for farmed shrimps in the world market, particularly in Japan, United States and Europe brought about the upsurge in the production of shrimps [1]. Virtually all farmed shrimp are penaeids, and just two species of shrimp - the *Penaeus vannamei* (Pacific white shrimp) and the *Penaeus monodon* (giant tiger prawn) -account for roughly 80% of all farmed shrimp [1]. Giant tiger prawn is the largest of all the cultivated shrimps; it can grow up to a length of 36 cm and is farmed in Asia. *Penaeus monodon* is the species of choice due to its high growth rate, significant tolerance to environmental stress, ease in reproduction, and its unquestionable market demand.

In shrimp farming, growth and production of shrimp species are depend on the population density [1]. It is a key factor for the farmers to determine the optimum stocking density of the animals being reared to maximize production and profitability. To gain high productivity, farmers stock higher shrimp density in ponds and practice over feeding. As a result, wastes as dissolved nutrients, uneaten feed and metabolic products are either directly or indirectly released to the surrounding environment, causing accelerated eutrophication. Under this culture condition, shrimps are vulnerable to many biotic and abiotic factors and often results in poor production [1].

Shrimp farming is prominent in Southwest coast of India, particularly in the Kollam coast. *Penaeus monodon* is the most commonly cultured penaeid shrimps in Kollam coast. Intensification of shrimp farms and unscientific management strategies overwhelmed the shrimp farming industry of Kollam [2]. Therefore, it is necessary to study the stocking density of shrimp in order to propose the optimal stocking density. As per the literature survey, so far no studies have been taken up in this area to investigate the effect of stocking density on the survival rate and growth of shrimp. In this context, the present study is designed to elucidate the influence of stocking density and water exchange rate on the survival, growth and apparent feed conversion ratio of *P. monodon.*

MATERIALS AND METHODS

Six experimental ponds (three for high density (HD) stocking and rest for low density (LD) stocking) located in Manroe Island, Southwest coast of India were selected for the culturing of *P. monodon* over a period of four months (~100 days). The ponds located near the brackish water canals are preferred for the study, so that relatively cleaner inlet water can be used. All

*Address correspondence to this author at the Department of Botany and Biotechnology, Sree Narayana College, Kollam 691001, South India; Tel/Fax: +91 9895530247; E-mail: sujansugathan@gmail.com

ISSN: 1814-8085 / E-ISSN: 1927-5129/14

the experimental ponds are earth filled embankment with an area of 1.3 acres and normal water depth of 1–1.5 m. Initially all the ponds were allowed to sun dry and crack to increase the capacity of oxidation of hydrogen sulphide and to eliminate the fish eggs, crab larvae and other predatory aquatics. The bottom of the ponds were then ploughed horizontally and vertically to a depth of 25 cm to oxygenate the bottom soil and to increase the fertility. After drying and tilling, the ponds were filled with tidal water. Thereafter, ponds were treated with tea seed cake (dosage rate of 50 Kg ha^{-1}) to kill unwanted aquatics and to produce blooming. After two days of tea-seed application, agricultural lime stones ($CaCO_3$) were applied at a rate of 60.2 Kg ha^{-1} in order to neutralize organic acid. The limestone was soaked in water, kept overnight and sprayed all over the farm in the subsequent day to control the pH level. After 2-4 days of liming the pond was applied with semi dried cow dung. The water colour of each pond was turned to light green after three days. Then the water level was raised and Urea, Triple Super Phosphate (TSP) and muster cake was added for improving pond productivity. The larvae of *P. monodon* (PL 20/ screened for white spot syndrome virus) were purchased from Matysafed Prawn Hatchery, Thirumullavaram, Kollam and transported in oxygenated double-layered polythene bags (Brine shrimp nauplii was given along with seeds during packing). The seeds were brought to the farm site and bags were kept in the pond water to adjust the temperature. The ponds were then stocked with animals that sourced from the same hatchery batch. The nursery area used for seed stocking was usually 5% of the total pond area. After that the pond water was added slowly into the seed bag to adjust the salinity and pH. Subsequently the seeds were released slowly into the nursery section. The average initial biomass and stocking density in high density ponds and low density ponds were appended in the Table **1**. Shrimps were fed with commercial feed (Charoen Pokphand Group) according to the rate and schedule as recommended elsewhere [3]. The feeds were dispersed by hand broadcasting over the water by using a boat criss-crossing the pond. Monitoring of water quality parameters such as temperature, pH and dissolved oxygen (DO) were carried out every two weeks until the end of the experimental period. During the culture period, brackish water was generally exchanged according to the following schedule: water exchange was not performed for the first 4 weeks and thereafter, the water was exchanged daily at a rate of 10-35% of pond volume in accordance with the stocking density. Sampling of shrimps was done fortnightly and the measurements of length and body weight of about 200 shrimps from the total stock in each pond was recorded to ascertain the growth rate. Shrimps were cultured for ~100 days. One day prior to harvest, water level in each ponds were lowered to maximum. On following days, shrimps were seined with a cast net and remaining shrimps were manually harvested from ponds. The shrimps harvested were counted and weighed en-masse. The survival rate, mean body weight, production rates per ponds and the overall Feed Conversion Ratio (FCR) were determined.

Hydrological Assessment

The water quality parameters (salinity, pH, temperature and dissolved oxygen) were recorded weekly using a water analysis kit (Hach Company, Loveland, CO). The measurements were taken by placing the specific probe deep into the each

Table 1: Details of Culture Trials in Brackish Water Ponds at Manroe Island, Kollam

Particulars	High density ponds			Low density ponds		
	1	2	3	4	5	6
Average size of the pond (acre)	1	1	1	1	1	1
Total No. of seed stocked	40,000	40,000	40,000	20,000	20,000	20,000
Stocking density (animals m^{-3})	12	12	12	6	6	6
Month and year of stocking	Dec-2010	Dec-2010	Dec-2010	Dec-2010	Dec-2010	Dec-2010
Initial biomass at stocking (g)	4.14	4.17	4.12	4.12	4.15	4.22
Feed	C.P.	C.P.	C.P.	C.P.	C.P.	C.P.
Culture days	~100	~100	~100	~100	~100	~100

experimental pond and allowing the reading to stabilize before recording the final reading. The water level was measured by using a standard scale with cm marking.

Microbiological Analysis

Microbiological analysis was carried out on shrimps collected in triplicate from each experimental pond during 10th, 50th and 90th day of feeding trial for one crop period ranging from 98 to 100 days. During transportation, samples were carried by thermo-cool box packed with ice to maintain the temperature ≤5°C. The bacteriological analysis commenced within 2 hr immediately after the samples had been collected. Randomly collected specimens were screened for the presence of *Vibrio* spp. Individual specimens were rinsed thoroughly in double distilled water and surface was sterilized with 60% ethanol. Thereafter, different body parts of shrimps were dissected out in sterile condition, pulverized in normal saline and swabbed on TCBS agar plates (Thiosulphate Bile Salt Sucrose agar, Hi-Media, Mumbai) in triplicate and incubated in an inverted position at 37°C for 24 hrs. *Vibrio* spp. was identified as characteristic discrete, round colonies which were yellowish / greenish in appearance [4].

Same experiment was repeated with water samples collected from the test shrimp rearing ponds. The water samples were sourced from a depth of 0.3 m below the surface using sterile screw-capped bottles of 50 ml capacity and brought to the laboratory within 2 hrs of sampling in an ice box. The collected samples were filtered through Whatman filter paper No. 1 and serially diluted in sterile brackish water, plated on TCBS and incubated in an inverted position at 37°C for 24 hrs.

Statistical Analysis

Statistical analyses were conducted using SPSS software for Windows version 11.2 (SPSS, Chicago, IL, USA). Differences were considered to be significant at the level of 0.05. All means were given with standard deviation (SD).

RESULTS

The experimental ponds were harvested after four months of culture. In the present study, the stocking density showed a clear impact on the survival rate, final body weight, specific growth rate, food consumption and FCR. Final survival rate and weight of shrimp in the LD (Low Density) experimental ponds were comparatively higher than those of HDP (High Density Ponds). Shrimps cultured under high density ponds exhibited low survivability, combined with high feed conversion rates (FCR). The comparative analysis on shrimp production data among farms with two different stocking densities were shown in Table **1**. The study revealed a continuous water quality deterioration (DO temperature, salinity and pH) and frequent mortality in HD ponds. In addition, production yield at harvest was significantly differed between HD and LD experimental ponds. Average production of HD and LD experimental ponds was 582 Kg and 439 Kg pond^{-1} (Table **5**).

Survival Rate

At the end of the experimental period, shrimps cultured at low stocking density exhibited an excellent mean survival rate than those at HD culture. Survival rates of shrimps at lower stocking density did not differ significantly. The survival rates of shrimps reared in LD experimental ponds were over 68.4% (Figure **1**)

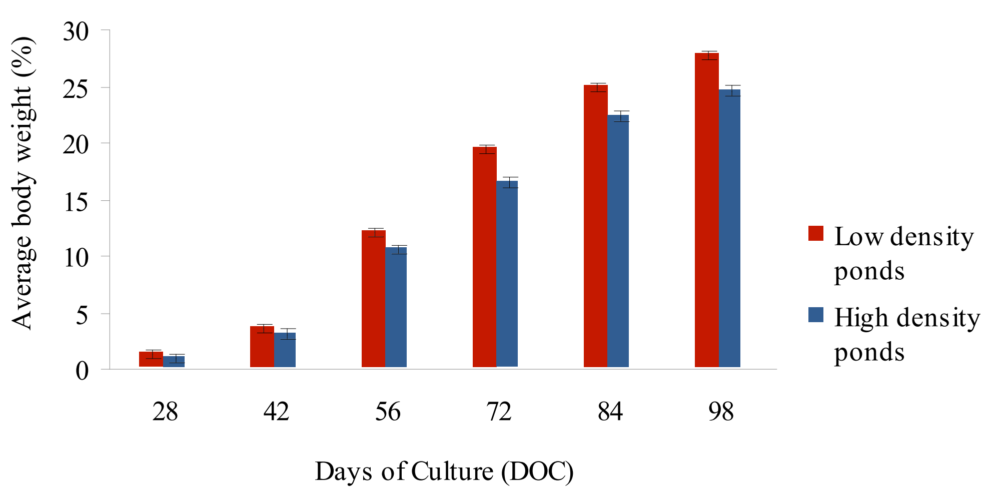

Figure 1: Average body weight of shrimps in low and high density ponds.

whereas, a significantly lower (51.7%) rate was observed at high stocking density experimental ponds.

Growth Performance

The growth increments in length and weight of shrimps under high and low density culture conditions were measured and results are presented in Figures **1** to **3**. It was found that growth rate negatively affected the higher stocking density. Maximum growth was observed in the shrimps cultured under low density ponds. Shrimps reared at low stocking density exhibited similar growth patterns throughout the culture period. At the time of harvest, average body weight and length of shrimps reared at LD experimental ponds were 27.8 g and 18.5 cm respectively. The cumulative weight and SGR of shrimps cultured in LD ponds were remarkably higher than those of HD ponds. No significant differences were found among the SGR of shrimps cultured at low density. Similarly, FCR values of shrimps in LD ponds were significantly better than those reared in HD experimental ponds. At the end of the culture, the average FCR value of shrimps reared at LD ponds was 1.8 whereas shrimps of HD ponds displayed a FCR value of 2.3. The average production rate per LD pond was 439 Kg whereas 582 Kg for HD pond.

Microbiological Analysis

The results of microbiological analysis were summarized in Table **2**. After 24 h of incubation under aerobic conditions, round yellow-green colonies with an increasing diameter of 1mm were appeared on the TCBS agar. The shape of colonies showed round, diameter was <1 mm and the colour were yellow and green. It was found that, the all the shrimp samples collected on DOC-10 was free of *Vibrio* spp. However, at DOC-50 the presence of *Vibrio* spp. in the samples of HD ponds was 5.4 x 10^4 CFU ml^{-1} and in the LD

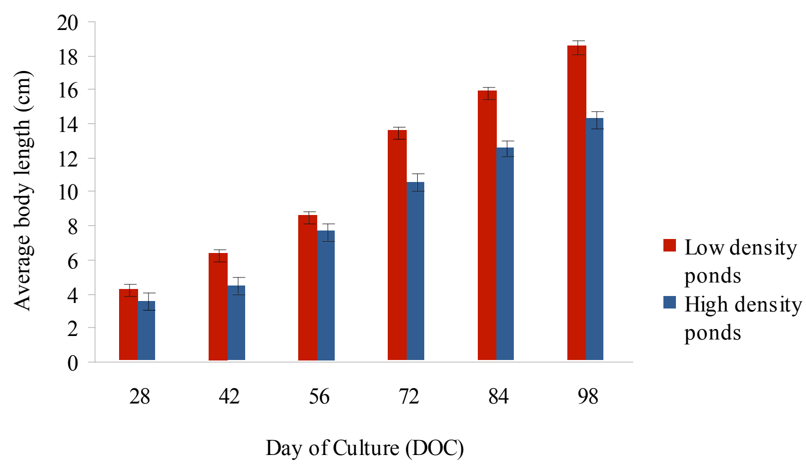

Figure 2: Average body length of shrimps in low and high density ponds.

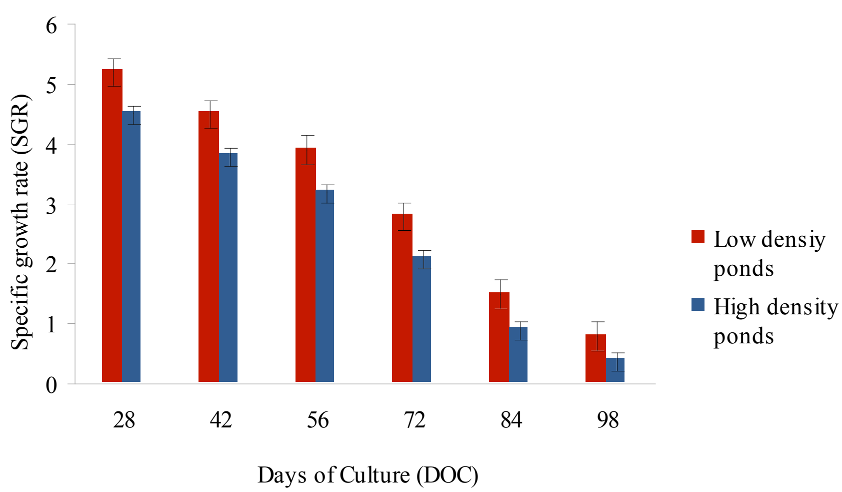

Figure 3: Specific growth rate of shrimps in low and high density ponds.

Table 2: Microbial Load in Low Density Ponds and High Density Ponds

DOC	Microbial load in low density ponds		Microbial load in high density ponds	
	Shrimp sample	Rearing water	Shrimp sample	Rearing water
DOC-50	8.8 x 10^2 CFU ml^{-1}	10^3	5.4 x 10^4 CFU ml^{-1}	10^4 to 10^5
DOC-90	2.4 X 10^3 to 7.8 x10^3 CFU ml^{-1}.	10^3	2.4 x 10^6 CFU ml^{-1}	10^6 to 10^7

CFU- Colony Forming Unit.

ponds *Vibrio* load was 8.8 x 10^2 CFU ml^{-1}. At DOC-90, *Vibrio* load sharply increased in the specimens collected from HD ponds (>10^5) whereas in the LD pond it ranged from 2.4 X 10^3 to 7.8 x10^3 CFU ml^{-1}.

During the period of first sampling, *Vibrio* counts were lower in all the tested ponds and not significantly differ between the ponds. However, during the mid culture period (DOC-50), higher *Vibrio* counts of 3.7 x 10^4 to 8.5 x 10^5 CFU ml^{-1} were observed in the high density ponds whereas in the low density ponds, the count was 2.8 X 10^3 to 5.6 X 10^3 CFU ml^{-1}. At the time of harvest, *Vibrio* counts of the high density ponds increased sharply to 5.7 x 10^6 to 1.8 x 10^7 CFU ml^{-1}.

Physio-Chemical Variables

During the experiment the physio-chemical parameters of the ponds such as salinity, dissolved oxygen, pH and temperature were analysed fortnightly (Table **3**, **4**). Physical and chemical variables showed significant differences among high and low density ponds. Water temperature and salinity along the experimental period ranged from 26.1 to 30.5˚C and 9.2 to 12.2 ppt without main or constant differences among the HD and LD experimental ponds; however, pH and DO appeared significantly differed among the experimental ponds (Table **3** and **4**).

DISCUSSION

The stocking density of shrimps is one of the vital zoo-technical factors that directly influence the survival, growth, behaviour, health, feeding, yield and profit [2]. In this study, stocking density of 12 PLs m/^{3} resulted in lower yield. There was a significant increase in the mortality with increased stocking density. The average survival rate during the culture period was 51.7% in high density ponds whereas 68.4% in low density ponds. No significant differences were observed on survival rate of shrimps between LD ponds. Several studies have evidenced that increased stocking density cause negative effect on survival and growth of shrimp

Table 3: Physiochemical Parameters of Low Density Ponds

Parameter	DOC 14	DOC 28	DOC 42	DOC 56	DOC 70	DOC 84	DOC 98
DO (mg L^{-1})	6.4±0.26	6.2±0.34	6.1±0.13	5.9±0.36	6.2±0.15	6.1±0.13	5.9±0.14
pH	7.2±0.53	7.5±0.78	7.2±0.53	7.4±0.14	7.2±0.62	7.1±0.34	7.3±0.29
Salinity	12.2±0.42	12.1±0.52	11.5±0.42	10.4±0.56	10.1±0.47	9.4±0.54	9.2±0.28
Temperature (°C)	26.1±0.47	28.7±0.81	30.2±0.38	29.8±0.67	28.6±0.58	28.2±0.65	28.1±0.13

Mean ± SD (*n*=3).

Table 4: Physiochemical Parameters of High Density Ponds

Parameters	DOC 14	DOC 28	DOC 42	DOC 56	DOC 70	DOC 84	DOC 98
DO (mg L^{-1})	5.7±0.04	5.3±0.16	4.9±0.25	4.3±0.29	4.1±0.21	4.3±0.17	4.1±0.15
pH	7.2±0.37	7±0.26	6.8±0.19	6.1±0.18	6.2±0.43	6.3±0.15	6.5±0.33
Salinity	11.9±0.27	12.1±0.41	11.5±0.35	10.8±0.05	9.7±0.54	8.9±0.84	8.5±0.36
Temperature (°C)	27.5±0.32	28.1±0.51	30.5±0.37	30.1±0.43	29.5±0.71	28.9±0.73	28.3±0.18

Mean ± SD (*n*=3).

Table 5: Average Harvest Details of *P. monodon* Cultured in Low Density and High Density Ponds

Particulars	Low density ponds (6 PLs m^{-3}) in Rs.	High density ponds (12 PLs m^{-3}) in Rs.
Pond area (acre)	0.8	0.8
Initial stock (Nos.)	20,000	40,000
Culture days	100	100
Average body weight	27.8±1.8	24.6±1.3
Survival rate	68.4±3.5	51.7±2.4
FCR	1.83	2.3
Total production (Kg)	439	582

[5, 6]. Similarly, increased stocking density may leads to an enhanced energy requirements causing reduced growth and food consumption [7]. This impaired growth due to high stocking density may be attributed to reduced food consumption, lower food conversion rate or increased metabolic cost [8-10]. Specific growth rate was higher in the initial phase of culture period and then declined continuously as the experiment progressed. Likewise, mortality rate was also higher in the first month of culture afterwards declined to a lower level. Moreover, higher stocking density will require more food inputs and thereby generates more wastes [11].

In the present study, a decreased survival of *P. monodon* with elevated stocking density was observed, which was similar to the results of Chakraborty *et al.,* [12] who reported *P. monodon* stocked at 8 animals/m^2 were showed increased mortality, lower weight gain and higher food consumption. In many studies, the growth and production of cultured species were observed to be dependent on the population density [13]. Therefore manipulation of stocking density is extremely important in maximizing the production [14]. According to Rodriguez *et al.*, [15] the growth rate and survival rates were inversely related to stocking density for *P. monodon* culture in the net enclosures. This was also supported by the results of Siddharaju and Menon [16], who reported the lower survival rate of 97% in the higher stocking density of 20 m^{-2} and the higher survival rate of 100% in the lower stocking density of 15 m^{-2} for the culture of *P. monodon* in the cages. Further, Tookwinas [17] reported the survival rate of 75% at a stocking density of 23 m^{-2} for the pen culture of *P. monodon*.

A high exposure to chemicals and biological products can increase the stress levels of the cultured shrimps and thereby decrease the growth rate and/ or increase their susceptibility to infections [18, 19]. The risk of diseases in shrimp farming often increases with culture intensity and high stocking densities [1]. High pond densities will facilitate the spread of pathogens between ponds [19]. With increased shrimp density, management becomes more difficult and the farmer may use antibiotics, chlorine, formaldehyde, etc. [20, 21] to kill both algae, bacteria and parasites. Antibiotics were used to prevent and treat *Vibrio* infections [22]. In India, mass mortality of shrimp larvae was due to the activity of antibiotic resistant *Vibrio harveyi* [23]. Therefore in the present investigation, *Vibrio* spp. was studied qualitatively in all culture ponds. It was found that samples collected from all the ponds during mid-culture period and end of the culture were positive for *Vibrio*. The results of the quantitative *Vibrio* counts of all the ponds stocked with two different densities of shrimps indicate that, the *Vibrio* count of water and shrimp samples are influenced by stocking density and their counts were increased with increasing stocking density and day of culture. It was observed that *Vibrio* count is always higher in every sample collected from high density ponds. As noted by Karunasagar *et al*., [24], the secondary infection of *Vibrio*s in *P. monodon* occurs due to stress, high stocking density, unstable environmental conditions and Virion particles.

Prevailing abiotic conditions during the experimental period were considered suitable, as temperature, salinity, DO and pH values in the LD experimental ponds varied within the tolerance ranges [25]. The physical and chemical characteristics of water in low density experimental ponds during the experiment were well maintained within the optimum levels recommended for *P. monodon* culture [4] whereas, it varied considerably among high density experimental ponds. The HD ponds managed with a daily water exchange rate of 35% and LD ponds managed with 15% per day. The dissolved oxygen and pH value exhibited significant difference between density treatments. Water quality parameters include dissolved oxygen (D.O.) and pH that commonly affected the yield in HD ponds. In all culture conditions, growth rate of the shrimps was higher during the first two months after stocking than in the later part of the experiment, probably because of lower dissolved oxygen and greater shrimp biomass towards the end. In this study, dissolved oxygen concentrations in HD ponds were lower than the optimum level of 4 ppm for shrimp growth. Low dissolved oxygen concentrations in HD ponds might be due to huge biomass of shrimps. Low oxygen level is a common problem in ponds with high stocking density that in turn increases disease susceptibility [26].

In conclusion, results of the overall study envisages that growth, survival rate, SGR and FCR of shrimps reared at low density ponds were higher and therefore a stocking density of 6 PL/m^3 is recommend for culturing *P. monodon* in Kollam coast of India under these experimental conditions.

REFERENCES

[1] Sugathan S, Manilal A, Selvin J. Development of novel probiotic for the management of shrimp Vibriosis. Scholars' Press publishers. ISBN 978-3-639-71304-6

[2] Shakir C, Manilal A, Jayakumari M, Sujith S, Selvin J. Current status of shrimp farming in the southwestern coast (Manroe Island, Kollam) of India. Iranica J Ener Environ 2010; 1: 287-292.

[3] Mohanty K. Feeding management and waste production in semi-intensive farming of *Penaeus monodon* at different stocking densities. Aqua Inter 2001; 9: 345-355. http://dx.doi.org/10.1023/A:1020421309414

[4] Manilal A, Sujith S, Selvin J, Kiran G, Shakir C, Gandhimathi G. Virulence of vibrios isolated from diseased black tiger shrimp *Penaeus monodon* Fabricious. J World Aquac Soc 2010; 41: 332-343. http://dx.doi.org/10.1111/j.1749-7345.2010.00375.x

[5] Penha-Lopes G, Rhyne AL, Lin J, Narciso L. Effects of temperature, stocking density and diet on the growth and survival of juvenile *Mithraculus forceps* (Milne Edwards A, 1875) (Decapoda: Brachyura: Majidae). Aquac Res 2006; 37: 398-408. http://dx.doi.org/10.1111/j.1365-2109.2006.01447.x

[6] Schram E, Van der Heul JW, Kamstra A, Verdegem MCJ. Stocking density-dependent growth of Dover Sole (*Solea solea*). Aquaculture 2006; 252: 339-347. http://dx.doi.org/10.1016/j.aquaculture.2005.07.011

[7] Hengsawat K, Ward FJ, Jaruratjamom P. The effect of stocking density on yield, growth and mortality of African catfish (*Clarias gariepinus* Burchell, 1822) cultured in cages. Aquaculture 1997; 152: 67-76. http://dx.doi.org/10.1016/S0044-8486(97)00008-2

[8] Jorgensen EH, Christiansen JS, Jobling M. Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). Aquaculture 1993; 110(2): 191-204. http://dx.doi.org/10.1016/0044-8486(93)90272-Z

[9] Li HW, Brocksen RW. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). J Fish Biol 1977; 11: 329-341. http://dx.doi.org/10.1111/j.1095-8649.1977.tb04126.x

[10] Vijayan MM, Leatherland JF. Effect of stocking density on the growth and stress-response in brook charr, (*Salvelinus fontinalis*). Aquaculture 1988; 75: 159-170. http://dx.doi.org/10.1016/0044-8486(88)90029-4

[11] Folke C, Kautsky N. Aquaculture with its environment: *Prospects for sustainability*. Ocean Coast Manag 1992; 17: 5-24. http://dx.doi.org/10.1016/0964-5691(92)90059-T

[12] Chakraborty SC, Paul AK, Hossain MA. Effect of stocking density on growth of tiger shrimp (*Penaeus monodon*) fed on commercial formulated diets. Bangladesh J Fish Res 1997; 1: 53-63.

[13] Backiel T, LeCren ED. Some density relationships for fish population parameters. In: The Biological basis of Freshwater Fish Production, Gerking SD (Eds.), Blackwell Scientific Publications. Oxford, 1967, 261-293.

[14] Suresh Babu CH, Shailender M. Effect of Stocking Density on Growth and Survival of Giant Tiger Prawn, f Giant Tiger Prawn, *Penaeus monodon* in Estuarine Backwater Areas in Estuarine Backwater Area. Inter J Bio-Pharma Res 2012; 2: 68-71.

[15] Rodriguez EM, Bombeo IT, Fukumoto S, Ticar RB. Nursery rearing of *Penaeus monodon* (Fabricius) using suspended (hapa) net enclosures installed in a pond. Aquaculture 1993; 112:107-111. http://dx.doi.org/10.1016/0044-8486(93)90162-R

[16] Siddharaju S, Menon VR. Experiments on shrimp farming in Kovalam backwaters of Tamil Nadu. Proceedings of the Symposium on Coastal Aquaculture 1982; 1:134-145.

[17] Tookwinas S. Pen culture techniques of marine shrimp in Thailand. Infofish Inter 1990; 2: 38-40.

[18] Le Moullac G, Haffner P. Environmental factors affecting immune responses in Crustacea. Aquaculture 2000; 191: 121-131. http://dx.doi.org/10.1016/S0044-8486(00)00422-1

[19] Kautsky N, Patrik R, Michael T, Troell M. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture 2000; 191: 145-161. http://dx.doi.org/10.1016/S0044-8486(00)00424-5

[20] Primavera JH. A critical review of shrimp pond culture in the Philippines. Rev Fish Sci 1993; 1: 151-201. http://dx.doi.org/10.1080/10641269309388539

[21] Barg U, Lavilla-Pitogo CR. The use of chemicals in aquaculture: A brief summary of two international expert meetings. FAO Aquaculture Newsletter. 1996; 14: 12-13.

[22] Graslund S, Holmstrom K, Wahlstrom A. A field survey of chemicals and biological products used in shrimp farming. Mar Pollut Bull 2003; 46: 81-90. http://dx.doi.org/10.1016/S0025-326X(02)00320-X

[23] Karunasagar I, Pai R, Malathi GR, Karunasagar I. Mass mortality of Penaeus monodon larvae due to antibiotic resistant Vibrio harveyi infection. Aquaculture 1994; 128: 203-209. http://dx.doi.org/10.1016/0044-8486(94)90309-3

[24] Karunasagar I, Otta SK, Karunasagar I, Joshua K. Applications of Vibrio vaccine in shrimp culture. Fish Chim 1996; 14: 205-254.

[25] Boyd CE. Water quality in ponds for aquaculture. Alabama Agricultural Experimental Station, Auburn University, Alabama, USA, 1990.

[26] Le Moullac G, Soyez C, Saulnier D, Ansquer D, Avarre JC, Levy P. Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp (*Penaeus stylirostris*). Fish Shellfish Immunol 1998; 8: 621-629. http://dx.doi.org/10.1006/fsim.1998.0166

Received on 05-05-2014 Accepted on 22-05-2014 Published on 03-06-2014

http://dx.doi.org/10.6000/1927-5129.2014.10.32