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FIRST PACIFIC WHITE SHRIMP, *LITOPENAEUS VANNAMEI* (BOONE, 1931) CULTURE IN PAKISTAN: EVALUATION OF OPTIMUM SALINITY LEVEL FOR THE GROWTH PERFORMANCE AND SURVIVAL IN THE HYPO SALINE AND HYPER SALINE CONDITION UNDER POND ECOSYSTEM

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ABSTRACT

The present study was conducted in the coastal pond ecosystems of Pakistan to evaluate the optimum salinity level for the best growth performance of a commercial shrimp, *Litopenaeus vannamei*. Total of 6 lac seed was randomly distributed in three ponds with an average initial body weight of 0.1±0.01 g. The shrimps were cultivated in three different ponds demarcated as pond 1, pond 2 and pond 3 with salinity levels of 8 ppt, 22 ppt and 42 ppt, respectively for 105 days. The pacific white shrimp were fed with 35.0% crude protein diet with feeding frequency of 4 and initially feeding rate of 10% biomass per day. The feeding rate adjusted according to shrimp biomass on a weekly basis. Final weight, specific growth rate, average daily weight gain, viscerosomatic index, hepatosomatic index and survival rates were found optimum at salinity level of 22 ppt compared with salinity levels of 8 ppt and 42 ppt (p <0.05). FCR was better at 22 ppt salinity compared with two other tested salinity levels (p <0.05). The 22 ppt salinity level resulted in optimum growth of *L. vannamei* which might be due to improved osmoregulation. Feeding frequency of 4, protein 35% and salinity of 22 ppt were found suitable factors for the growth performance of *L. vannamei* reared in aquaculture system.

Key words: *Litopenaeus vannamei*, salinity, growth, survival rate, aquaculture.
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INTRODUCTION

Aquaculture is the highest-growing food sector in the world and plays a vital role in food and nutritional security (Kumari *et al*., 2018). A food crisis is expected in our century due to the rapidly increasing population, climate change, dwindling land and a decline in the marine resources. Aquaculture may serve as an alternative source of protein for over-exploited wild fish populations (Mavraganis *et al*., 2020;Hassan *et al*., 2020a). The white leg shrimp, *Litopenaeus vannamei* (Boone, 1931) is also known as king prawn is the important commercially significant euryhaline shrimp (Alcivar *et al*., 2007). This species has sustained demand in both domestic and export markets (Zulkarnain *et al*., 2020). Pacific white shrimp is globally cultured in China, Thailand, America, Malaysia, Indonesia, Peru, Colombia, India and Philippines (Saoud *et al*., 2003;Wurmann *et al*., 2004; Cheng *et al*., 2006;FAO, 2020). It tolerates a wide range of salinities and has been grown in the marine, brackish, estuary and freshwater ecosystems (Jaffer *et al*., 2020) *L. vannamei* is appropriate for intensive aquaculture (Han *et al*., 2018a).

In the face of worldwide variation, recognizing and envisaging the impact of manifold stressors is one of the critical problems for the protection of species (Cote *et al*., 2016;Hassan *et al.,* 2020b). Salinity is a significant environmental parameter in terms of shrimp physiology, altering food intake and efficiency of growth in shrimp species (Brito *et al*., 2000). Optimal salinity required for excellent growth (Bray *et al*., 1994). Euryhaline species tolerates hard environmental conditions but reduce growth performance (Abella *et al.*, 2013), survival (Kroeker *et al.,* 2013) and osmoregulation (Smith, 2003; Smith and Schindler, 2009). High salinity causes stress to increase the levels of Na+-K+-ATPase subunit and CA transcripts in gills can reduction the levels of trypsin and chymotrypsin transcripts in the hepatopancreas. Thus results in physiological stress, affect growth performances, morphological and physiological effects on species (Gao *et al*., 2011; Madeira *et al*., 2015; Grizzetti *et al*., 2017; Jaffer *et al*., 2020). Due to the diverse nature and various mechanisms of action, these stressors can result in additive, synergistic or antagonistic effects on survival, fecundity, metabolic and growth rates (Crain *et al*., 2008). White leg shrimp performed excellent growth in optimum hydrological parameters, such as pH 7.0-9.5 (Han *et al*., 2018b), the salinity of 5-35 ppt (Maicá *et al*., 2014;Wang *et al*., 2016). Ammonia originating from the excretion of cultivated shrimp and the ammonification has one of the most common toxins in aquaculture systems with uneaten feed or organic detritus. Accumulation of ammonia may lead to a decrease in water quality and risk of shrimp's production (Jiang *et al*., 2014).

Pacific white shrimp is able to survive in waters with salinity ranging from 1 to 50 ppt and can be used as a model species for studying osmoregulation (Kidder *et al*., 2006) and salt adaptations (Saoud *et al*., 2003). Optimum salinity is excellent for growth rates, feed conversion, gastric evacuation and survival of *L. vannamei*. It is worth mentioning that no appropriate information is available earlier on this aspect in Pakistan. To acquire more knowledge on the growth, amplify mechanism and also to deliver a scientific procedure for physiochemical parameters management, the present study was aimed to evaluate the optimum salinity level for the best growth performance of *L. vannamei* in the ponds culture system of coastal area in Pakistan under hypo-saline and hyper-saline environment.

MATERIALS AND METHODS

Experimental design: This study was designed in pond ecosystem in the coastal area of Sindh, Tatha, Pakistan (N: 24.546766, E: 67.46096) by following CRD (complete randomized design). Larvae of Pacific white shrimp were imported from Thailand SY AQUA (Thailand) commercial hatchery. In order to reduce the stress of larvae, oxygenated double-coated polythene bags were used for transportation. Ice packages also used between the internal and external coverings of polythene bags to sustain optimal temperature during the shrimp seed transportation. Acclimatization was performed before the experimental period. Seeds were taken to the pond and bags were kept in water for a while to adopt the seeds. Water was then applied to the PL bag gradually to maintain salinity and pH. The shrimps were released into the ponds (area: 1.5-hectare) slowly at a depth of 4 ft. A total number of 6 lakh individual with 0.1±0.01 g mean initial weight was randomly distributed into a pond containing 8 ppt, 22 ppt, and 42 ppt salinity separately. The formulated feed with 35% protein was initially fed at a rate of 10% biomass per day, further adjusted according to shrimp biomass on a weekly basis and four feeding frequency per day was maintained (Table 1). Circular trays were used for feeding. Based on shrimp biomass and survival, feeding rations and feeding time were altered on a weekly basis. Physicochemical parameters were tested from 3 experimental ponds on a daily basis. Cast net was used for sampling to track the health and growth of shrimp on a weekly basis to quantify the various parameters. An aerator was installed to sustain the level of dissolved oxygen (DO). Waters of ponds were exchanged at 30% of total volume twice a week. Larvae were cultured for a period of a total of 105 days.

Table 1. Feed formulation (g/100 g) and chemical analysis (%) of the applied diet.

Ingredients	Experimental diets (g/100 g)
fish meal	18.0
Casein	10.0
Gelatin	2.50
Squid liver meal	5.0
Soybean meal	4.0
Wheat flour	34.5
Fish oil	2.0
Starch	20.0
Vitamin/mineral premix4	2.0
Choline chloride	1.0
Lecithin	1.0
Chemical composition	**(% dry matter)**
Dry matter	88.4
Crude protein	35.8
Crude ash	5.15
Crude lipid	8.18

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

Pond preparation and fertilizers: Restoration of all ponds were done through the discharge of water and kept 25 days for drying. To eliminate heavy metal, toxins and accumulated feed in the bottom that cause diseases, hurrying organic matter decay and oxidation of abridged amalgams 4-7 cm of soil was removed from each pond. Liming was carried out to nullify the acidity of the soil and increase overall alkalinity and total hardness concentration that enhance primary food productivity in shrimp pond. Organic fertilization increased zooplankton directly on manure particles. Enabling a rapid increase in zooplankton abundance in dry pond manure was distributed on the pond floor before filling with water. Inorganic nitrogen and phosphorous fertilizers were applied in shrimp ponds to stimulate phytoplankton, photosynthesis to produced primary productivity to increased zooplankton. Dolomite was used to help in plankton development.

Physicochemical parameters: The hydrological parameters such as ammonia, temperature, pH, salinity, and DO were measured daily at 6 am, 2 pm, 8 pm and 12 am. The dissolved oxygen was measured with DO-meter

(Model: HI9146). Salinity was measured by using hand refract meter, temperature ºC with thermometer, pH by using a calibrated (pH -3 model) and ammonia was analyzed by using the SDM (Solorzano, 1969). Phosphate (mg/l) was measured according to Aminot and Chaussepied (1983). TSS (mg/L), and ammonia, alkalinity, nitrite nitrate, and nitrite were investigated by using the method described by APHA (1995).

Experimental formulation and statistical analyses: The influence of salinity on WG (weight gain), ADWG (average daily weight gain), SGR (specific growth rate), FCR (feed conversion ratio), HSI (hepatosomatic index), VSI (viscerosomatic index), CF (Condition factor) and survival rate were analyzed among the salinity-related ponds of 8 (pond 1), 22 (pond 2) and 42 ppt (pond 3). Sampling was performed on a weekly basis (Fig. 1). At the time of each sampling, the shrimp length, weight and survival were calculated (Szkudlarek and Zakes, 2007; Hossain *et al*., 2012;Hassan *et al.,* 2020b; Sabbir *et al.,* 2020). Indices for the evaluation of growth performance have been determined as follows:

Weight gain = Final Weight-Initial weight

Average daily weight gain = {(Final weight-Initial weight)/Days}

Specific growth rate (%) = {(Final Weight-Initial weight)/Days} ×100

Feed conversion ratio = Total feed (g)/ Weight gain (g)

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

Condition factor = (Weight/Length^3) ×100

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

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

Fig. 1. Pond preparation (upper left), culture pond (upper right), sampling by cast net (lower left) and collected white shrimp individual (lower right) for average body weight and survival.

Normality of data was checked through GraphPad Prism. Water quality variation and growth rate between treatments were checked by ANOVA (as pass normality) and followed by post-hoc Tukey's numerous range test by using SPSS 17.0 software. The significance level was set at $p<0.05$.

RESULTS

Physicochemical parameters: Hyper saline condition increased ammonia concentration, DO and pH and decreased carbon dioxide during the daytime while in night time DO increased and pH decreased. The dissolved oxygen, carbon dioxide and pH in the ponds change relative concentrations over 24 hours, respectively. The foundations for alkalinity react to acids and neutralize them. Both acids and bases were reacting to the bicarbonates and carbonates, and pH variations were minimized. (Table 2).

Morphological indices, growth and survival: The growth performance: final body weight, weight gain, specific growth rate, average daily weight gain, feed conversion ratio of *L. vannamei* at different salinities were given in Table 3. The highest FBW (25.40±0.64 g) was recorded in the pond (2), salinity at 22 ppt. The other two groups had lowered *i.e.*, 8 and 42 ppt salinity.

Table 2. Water quality parameters (Mean±SD) of the Pacific white shrimp, *Litopenaeus vannamei* culture at various salinities over an experimental period of 105 days.

Parameters	Pond 1	Pond 2	Pond 3
Dissolved oxygen (mg/l)	6.5±1.40[a]	5.5±2.11[b]	7.0±1.20[a]
Temperature (°C)	31.2±3.20[a]	30.1±1.80[a]	30.2±3.10[a]
pH	8.2±1.05[a]	7.5±2.11[b]	5.30±1.20[c]
Alkalinity (m/l)	120±2.11[a]	125±1.11[b]	120±3.22[a]
Nitrite (mg/l)	0.09±0.01[a]	0.09±0.10[a]	0.08±0.02[a]
Nitrate (mg/l)	0.99±0.08[a]	1.20±0.09[b]	1.41±0.11[c]
Phosphate (mg/l)	0.30±0.07[a]	0.40±0.1[b]	0.41±0.2[b]
Total ammonium nitrogen (mg/l)	0.03±0.0.01[a]	0.06±0.02[b]	0.12±0.04[c]
Total suspended solids (mg/l)	560.66±8.88[a]	610.20±6.78[b]	740±35.44[c]

Mean of 3 replicates ±SD, in the row, different letters designate significant variances ($p<0.05$)

The SGR (5.45±0.06) attained significantly higher ($p<0.05$) after 105 days in the pond 2 at 22 ppt salinity while the other ponds were 5.09 ± 0.04 (pond 1, 8 ppt) and 4.90± 0.50 (pond 3, 42 ppt). However according to the broken line model SGR of *L. vannamei* at 22 ppt salinity exposed best growth performance compared to other groups (Fig. 2). Relatively high significant values for ADWG were also recorded in pond 2 (22 ppt salinity) further increases or decreases salinity reduces the ADWG (Table 3). In this experiment, FCR was significantly affected by the salinity levels. *L. vannamei* at the 22 ppt salinity pond 2 exposed a significantly better FCR of 1.11±0.34 ($p<0.05$).

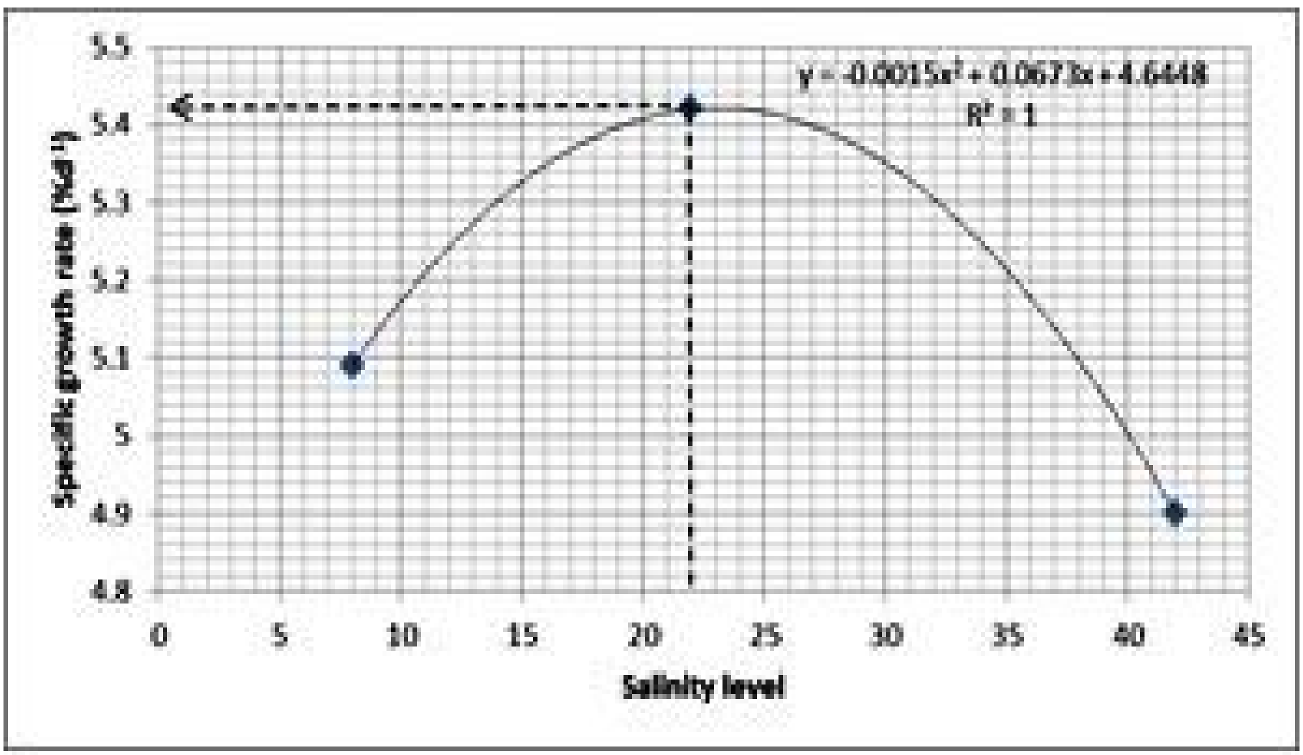

Fig. 2. Optimal salinity level of *Litopenaeus vannamei* based on specific growth rate (SGR% per day) as determined by the phenomenal regression.

Hepatosomatic index and Viscerosomatic index of white leg shrimp at salinity 22 ppt were significantly higher ($p<0.05$) than the other two groups of 8 and 42 ppt salinity. Better condition factor recorded in 22 ppt compared to other groups. The highest survival (90.70±2.17) was recorded at 22 ppt salinity in pond 2 which was significantly different from that of 8 ppt salinity in pond 1 and 42 ppt salinity pond 3 (Table 3).

Table 3. Growth rates of Pacific white shrimp, *Litopenaeus vannamei* cultured at different salinities over 105 days of the study period.

Biotechnical parameters	Salinity level		
	Pond 1 (8 ppt)	**Pond 2 (22 ppt)**	**Pond 3 (42 ppt)**
Initial body weight	0.1±0.01[a]	0.1±0.00[a]	0.1±0.01[a]
Final body weight (g)	18.30±0.22[a]	25.40±0.64[b]	15.12±0.68[c]
Specific growth rate (%d^{-1})	5.09 ± 0.04[a]	5.42 ± 0.06[b]	4.90± 0.50[c]
Final body length (cm)	14.33±0.30a	15.9±0.20[b]	13.10±0.40[c]
Feed conversion ratio	1.32±0.12[a]	1.11±0.34[b]	1.40±0.01[a]
Average daily weight gain (g/day)	0.17±0.00[a]	0.24±0.01[b]	0.14±0.01[a]
Weight gain (g)	18.20±0.90[a]	25.30±0.80[b]	15.11±02[c]
Hepatosomatic index	3.20±0.1[a]	3.31±0.2[a]	3.04±0.1[a]
Viscerosomatic index	4.10±0.3[a]	4.60±0.2[b]	3.80±0.2[c]
Condition factor	0.62±0.00[a]	0.63±0.00[a]	0.67±0.00[a]
Survival (%)	80.20±3.20[a]	90.70±2.17[b]	70.51±2.88[c]

Values are the mean ± SD of triplicate groups in the same row with different superscripts are significantly different (p<0.05).

DISCUSSION

Water quality plays a vital role because it has a direct effect on the general health status of cultured shrimp (Xu *et al*., 2018). High salinity affects the feed conversion ratio (Leal *et al*., 2019). Highest of ammonia were created by shrimp at 42 ppt salinity. The white shrimp under recommended ammonia concentration were recorded for juveniles who were in hypo-osmotic conditions 8 and 22 ppt (Jiang *et al*., 2014). Increased level of ammonia is also causing mortality. DO, pH, temperature and alkalinity were within suggested ranges for the culture of pacific white shrimp (Venkatachalam *et al*., 1974; Whetstone *et al*., 2002;Maicá *et al*., 2014; Kumari *et al*., 2018). The *L. vannamei* has adopted a broad salinity range from 0.5 to 42 ppt (Walker *et al*., 2009), which is hyper-osmoregulator and hypo-osmoregulator when the ambient salinity is above and below the isotonic point of 718mOsm kg^{-1} (equivalent to 25 ppt) (Gao *et al*., 2011). Growth performance, WG, ADWG were observed to be relatively higher (p<0.05) in 22 ppt compared to 8 and 42 ppt. In terms of SGR, 22 ppt salinity produced a higher rate while there was no significant difference (p>0.05) at 8 and 42 ppt and similar with the findings of Jaffer *et al*. (2020). White leg shrimp reared at 35 ppt salinity higher growth rates compared to 49 ppt salinity (Palafox *et al*. 2007; Sui *et al*., 2015;Figueroa *et al*., 2017). Various studies have focused on optimizing salinity for its growth performance (Palafox *et al*., 1997). Several of them suggest that shrimps had optimal growth performance near the isotonic stage. (Bindu and Diwan, 2002;Ayaz *et al*., 2015). The hyper-saline water effects on growth rate (Ogle *et al*., 1992;Samocha *et al*., 1998;Silva *et al*., 2010), oxygen consumption (Lemos *et al*., 2001), histological changes of hepatopancreas (Li *et al*., 2008) and survival (Li *et al*., 2007). Shrimp at the extreme of their salinity tolerance range often exceed their ability to osmoregulate. In the present study, 22 ppt salinity was optimal for growth and survival of white leg shrimp and significantly higher than the salinity of 8 and 42 ppt, similar findings were also reported by Chen *et al*. (1995), Zhang *et al* (1999) , Li *et al*. (2007) and Jaffer *et al*. (2020),

In the present study, the highest survival was recorded at 22 ppt that was significantly different from 8 and 42 ppt salinity. This conclusion is the same as Samocha *et al*. (2004). In this study, white leg shrimp survival was significantly affected by salinity, with enlarging mortality as salinity increasing or decreasing from optimum level. A related finding was observed by Laramore *et al*. (2001). In a study with low-salinity of 8 ppt observed lower survival and increase in salinity (8 to 22 ppt) improved survival of *L. vannamei* (Roy *et al*., 2007) further, increase salinity to reduce growth and survival (Sui *et al*., 2015;Jaffer *et al*., 2020). In this study, FCR was highest in the Pond 2 at 22ppt salinity while the lowest was recorded in a pond 1 at 8 ppt and pond 3 at 42 ppt. The enhancement in feed utilization with cumulative salinity to the optimum range was also established by important rise in protein retaining rate of *L. vannamei reared* at 22 ppt salinity associated with shrimp sustained at 42 ppt and 8 ppt (Decamp *et al*., 2003; Maicá *et al*., 2014;Jaffer *et al*., 2020). White leg shrimp had the greatest HSI and VSI at 22 ppt salinity which differs from that of 8 and 42 ppt. Salinity did not effect on CF (Wang *et al*., 2014;Ali *et al*., 2017). Such findings were compatible with the observations for other shrimp species (Raj and Raj, 1982). The optimum salinity for *Penaeus stylirostris* was 24 ppt and *Litopenaeus setiferus* 26.8 ppt (Rosas *et al*., 1999). Optimum salinity determined for *L. vannamei* by Gong *et al*. (2004), Diaz *et al.* (2001) and Castille and Lawrence (1981). *Penaeus monodon* (Ferraris *et al.,* 1986) have optimum growth in 25-26 ppt. have optimum growth. *Penaeus chinensis* reared between 40 to 100 ppt salinity but optimum salinity is 25 ppt

(Chen and Lin, 1994) and *F. subtilis* in 14 ppt (Silva *et al*., 2010). The level of stress and physiological adaptation of *L. vannamei* to specific salinities can be regulated by means of their osmoregulatory ability, which is the alteration among hemolymph and intermediate osmolality (Daun *et al*., 2018a,b).

Hyper-regulation is the typical mode of shrimp power, primarily at salinity below 22 ppt, whereby hemolymph osmolality begins to be sustained aggressively above the underlying sea water. For salinities below 22 ppt, shrimps' function as strong, modest or low osmoregulation.

Conclusion: Salinity is one of the greatest fundamental ecological parameters antagonistic, synergistic effects on pacific white shrimp survival, fecundity, metabolic, physiology and growth performance. The optimum salinity level for the best growth performance of the *L. vannamei* in the coastal marine ecosystem of Pakistan was found as 22 ppt, but overall experiment indicted that 22.0± 14.0 ppt salinity have a pronounced effect on shrimp growth and survival which could be due to improved osmoregulation. This research is very important in providing useful knowledge for the *L.vannamei* to increase mass production and promote economic growth. This research would be very useful in providing appropriate cultural techniques to improve the production of *L. vannamei* and promote economic growth for the aquaculture sector.

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