AMMONIA AS WATER QUALITY PARAMETER IN ZERO SALINITY CULTURE OF *L.VANNAMEI IN EARTHEN* PONDS IN EPURUPALEM AREA, CHIRALA, A.P

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PRAKASAM DISTRICT, ANDHRA PRADESH

FEBRUARY 2020

DECLARATION

We declare that this thesis entitled "Ammonia As Water Quality Parameter In Zero- Salinity Culture Of Litopeneus Vannamei"is composed by us and has not been published or submitted in part or in full for award of any degree.

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V. Elisha Babu

S.L Sahithi

CERTIFICATE

This is to certify that the work incorporated in this thesis entitled, "AMMONIA AS WATER QUALITY PARAMETER IN ZERO-SALINITY CULTURE OF LITOPENEUS VANNAMEI" is the bonafide work carried out by **my students** under our supervision.

K Bhanu Prakash

B Ashok Kumar

Research Supervisors

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CONTENTS

AMMONIA AS WATER QUALITY PARAMETER IN ZERO SALINITY CULTURE OF *L.VANNAMEI EARTHEN* PONDS IN EPURUPALEM AREA, CHIRALA, A.P

Introduction:

Shrimp aquaculture sector occupies very important role in the socio-economic development of the country and also provide protinacious food for the poor people. The aqua industry has expected to account progressively for the insufficient aquatic food supply that would occur for the population increase expected until 2030 and it is the fastest growing food production sector in the world increasing with an average rate of 9.2% over the past 30 years (FAO 2005) which makes aqua industry one of the promising industry to meet future food demand.

In the recent years aquaculture intensification has become a common practice throughout the world. Farmers are reporting with higher stocking densities, artificial fertilization of the ponds and supplementary feeding using artificial feeds to get the maximum profit from a unit area. There is always a chance of stress to the growing organism with the over intensification. Under stress the pathogens present in the pond may enter and cause disease resulting in severe mortality. During the last few years Asian countries were severely affected with many viral diseases and faced massive economic losses particularly due to continuous outbreak of White Spot Disease (WSD). In India outbreak of WSSV to tiger shrimp *Penaeus monodon* has spread and caused large scale mortalities and severe damage to shrimp aquaculture industry. The pacific white shrimp *Litopenaeus vannamei* has become the main crustacean species produced through culture, with production exceeding that of tiger shrimp *Penaeus monodon* since 2003. The production of this species has been increased from 186,113 tons in 1999 to over 2.3 mmt in 2007.

Many studies have aimed to increase the shrimp production through manipulating of stocking density, fertilization, artificial feeding and opening of new lands for culture and combination of different species into culture system (Varghese et al., 1975; Chakraborti et al., 1985; Krishna, 2006). In practice, the densities at which farmers keep their stock are based on the experience and institution with codes of practice and hand books being used as guide. Information regarding effect of stocking density of the shrimp performance during intensive culture is limited, inconsistent and some time controversial.

Pacific white shrimp, *Litopenaeus vannamei*, is one of the most intensively cultivated shrimps all over the world (Perez Farfante and Kensley 1997) because of the reduced risk of catastrophic diseases and favorable environmental conditions (Boyd 2002; Zhu et al., 2006). Several authors described about the growth in shrimp culture systems based on stocking density (Cailout et al., 1976; Sedgwick 1979; Maguire and Leedow 1983) and some authors have reported an inverse relationship between growth and stocking density (Lee et al., 1986; Sandier et al., 1987; Whay-Ming and Yew-Hu, 1992; Daniels et al., 1995). No proper research has yet been done on the effect of stocking density in long term survival and growth performance of *L.vannamei*. Hence it was aimed to evaluate the effect of different stocking densities on the survival and growth of *L.vannamei* for the present study.

Success of aquaculture depends on providing animals with a satisfactory environment (Boyd and Tucker, 2009). Over the past few decades, shrimp farming in India has expanded rapidly to a vibrant export industry with the export production of 3, 57,505 MT worth USD 3.7 Billion (MPEDA, 2015) for 2014-15. The corresponding figures for 2015-16 in USD are 3.1

Billion (MPEDA, 2016). Given the ever-increasing consumer demand, high foreign exchange earning potential and stagnation in the wild catch, the shrimp farming has been expanding at phenomenal proportions. . The world needs an extra 40-60x106 tons of food fish by 2020. Therefore culture is transforming rapidly into an intensive type of semi-intensive mode. Growth and development of aquaculture should be sustainable. The growing aquaculture industry is haunted by a number of environmental and social issues.

Indian Aquaculture Scenario:

- As per GLOBEFISH, India is the fourth largest exporter of seafood in 2017
- Largest supplier of frozen shrimp to USA
- 2nd largest supplier of shrimp to European union
- Second largest supplier of frozen shrimp to Japan
- Largest supplier of cephalopods to EU
- Statutory body under the Ministry of Commerce & Industry, Govt. of India; set up in 1972 by an act of parliament.
- Nodal agency for promotion of the export of marine products
- Entrusted with the overall development (including infrastructure), regulation, and promotion for the export of marine products.
- Capture fisheries contribute about 45.18% of seafood export of India value and about 68.51% in quantity.
- The rest is contributed by coastal aquaculture, especially shrimps.

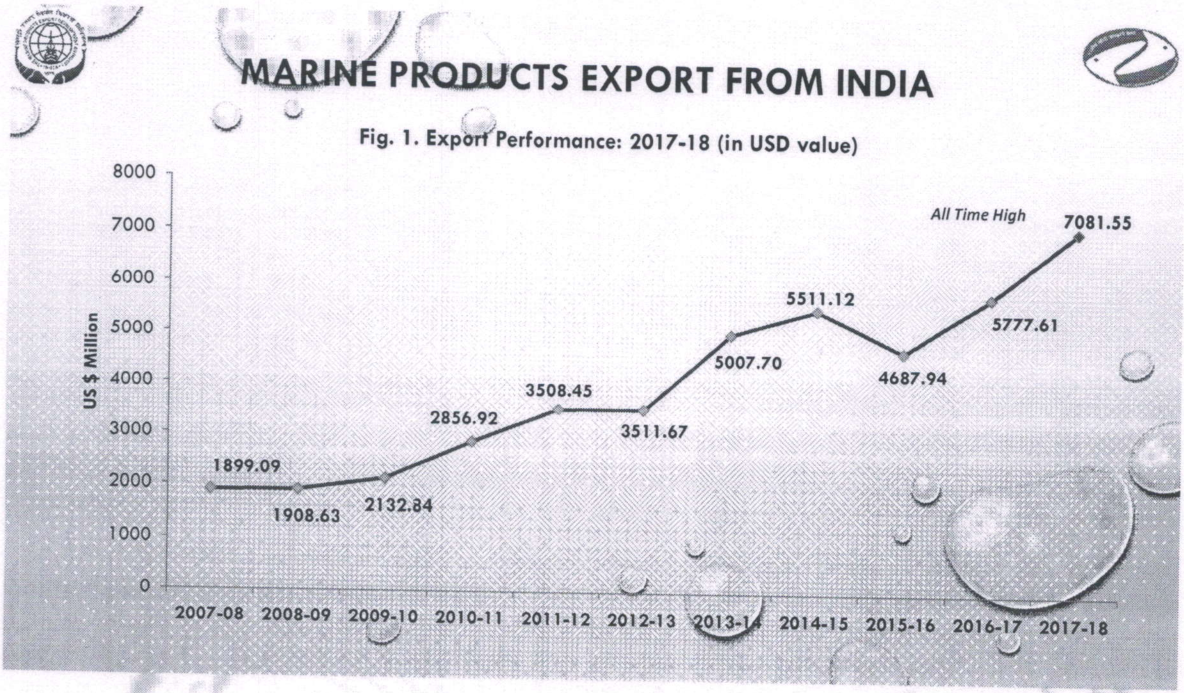


Fig. 1. Trends in progress of aquaculture production of export oriented species

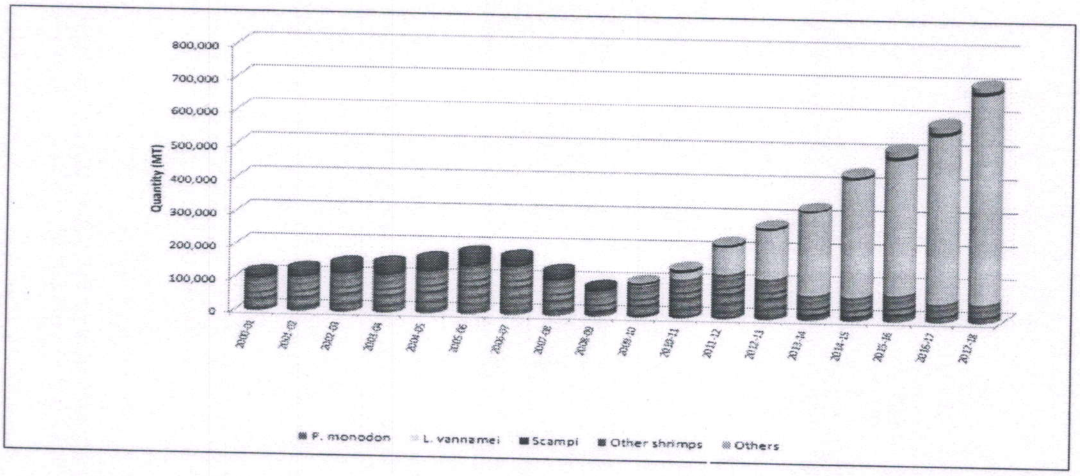
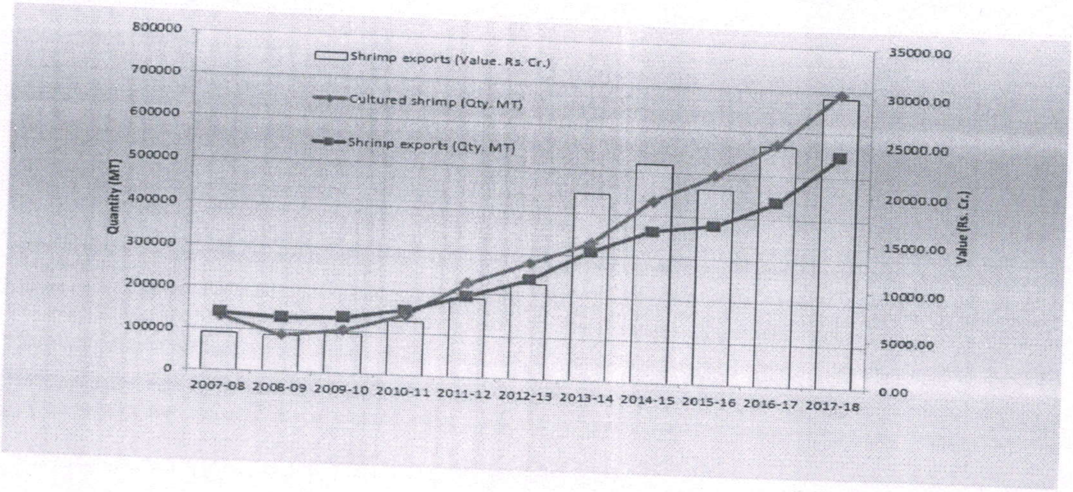


Fig. 2. Trends in progress of cultured shrimp production and shrimp exports



Shrimp Production in species-wise during 2017-18

Species	Production (in MT)
	Country Total
P. monodon	58,163
L. vannamei	5,01,297
F. Indicus /other shrimp	1,675

Total Shrimp Production in the Country during 2017-18

Sl. No.	State	Area Utilised(ha)	Production (Mt)	Productivity (Mt/ha/year)
1	West Bengal	52,131	71,051	1.36
2	Odisha	8,574	29,296	3.42
3	Andhra Pradesh	64,266	3,55,956	5.54
4	Tamil Nadu	8,751	49,054	5.60
5	Kerala	4,452	*3,987	0.90
6	Karnataka & Goa	1,154	*2,197	1.90
7	Maharashtra	1,652	6,842	4.14
8	Gujarat	5,656	42,755	7.56
	Total	1,46,636	5,61,138	3.83

including the production of F. indicus

47

Litopenaeus vannamei (white leg shrimp) species, which have been introduced to many coastal states of India, now account for 90 percent of the country's total shrimp culture. The species exhibits a fast growth rate and its culture period is significantly shorter than that of *Penaeus monodon* (tiger prawn), making it an attractive alternative to tiger prawn production in several countries.

Specific pathogen free (SPF)

Aquatic animals that have been produced and are tested and held under rigorous conditions of bio-security that provide assurances that they are free of certain specified pathogens.

- First SPF shrimp was developed on the big island of Hawaii, in Kona, in 1990.

- SPF animals offer an advantage to a country introducing a species for the first time as it offers some assurance that the imported animals will not introduce the listed pathogens to native species.

- Use of SPF shrimp has greatly reduced disease incidence and thus enhanced the shrimp production at global level.

- Use of SPF broodstock has reduced the spread of shrimp diseases worldwide and eliminated the industry's practice of capturing shrimps from the wild for seed production.

Aquaculture in Andhra Pradesh:

Andhra Pradesh is achieving rapid progress in aquaculture with vast potential for the development of fish and prawn cultivation and sea food production. The fish and prawn production has 6.4 per cent share in the Gross State Domestic Production (GSDP) and providing livelihood to 14.5 lakh population. Up to December 2017, the fish and prawn production achieved 27.49 lakh tonnes with GVA of Rs.34,041 crore (constant prices). During the year 2017-18, the State government had set the target of producing 33.84 lakh tonnes of fish and prawns with GVA of Rs.42,110 crore with growth rate of 22.35 per cent on production and 35.65 per cent on GVA.

Andhra Pradesh has a coast line of 970 km with vast scope for production of fish, prawn and other sea products. Keeping in view of huge demand for sea food in the international market, the state government is promoting the best practices like simplifying the procedures for registration of aqua farms through Mee-seva, permitting aquaculture in DKT lands, cluster approach and continuous awareness campaign at the primary producer level in the existing 181 aqua clusters covering 1.27 lakh hectares areas.

Andhra Pradesh has lion's share in the sea food exports from our country with 45 per cent share in the year 2016-17. Sea food worth Rs.17, 000 crore was exported from the state in the year 2016-17 against the total exports of worth Rs.37, 871 crore from India.

The present study was conducted to see if the stocking densities were having any impact on the growth performance of the shrimp grown in fresh water earthen ponds in an area where the culture of brackish water shrimp is new.

Ammonia and its effects in shrimp farming:

Ammonia is one of the most harmful water quality parameters and also a very toxic inorganic nitrogen compound for shrimp along with nitrite in aquaculture ponds. It exists in two forms in the pond water. One is unionized ammonia which is very toxic for the culture animals and the other is the ammonium ion which is not toxic. Both the substances constitute TAN which is used to calculate the former toxic form making use of a prescribed formula from TAN.

Ammonia is a natural compound that occurs in the environment. But it is also a by-product of industrial pollution, domestic and agricultural run-off. Fertilizers like Urea, Nitrogen fertilizers like Ammonium Sulphate, Ammonium phosphate etc add nitrogen to the pond. Uneaten feed, excreta of the culture animals are decomposed by the microbes and release ammonia. As the feed input increases so the ammonia concentration increases in the pond.

Daily fluctuations of Ammonia in the Aquaculture Ponds:

The concentration of ammonia is dependent on the pH and temperature of the pond water. The concentration goes up if the pH and temperature increase. The concentration of ammonia is measured high at low salinities and constant pH and temperatures. Usually, pH will be lowest in the morning and highest in the afternoon. The ammonia shows most toxic effect at pH above 8.0. The pH and temperature vary in a day and also the NH_3. Exposure to high ammonia for few hours is not harmful (Hargreaves & Kucuk). The LC50 values of ammonia for marine shrimp range from 0.7 to 3.0 ppm (Van Wyk et al, 1999). The safe concentration for long term exposure ranges between 0.05-0.15 ppm. The 96 hour LC50 for *L.vannamei* ranges between 1.20 and 2.95 ppm.

Table 1.2: Dependency of Ammonia on Temperature & pH

Temperature / **pH**	**16**	**18**
7.2	0.004	0.005
7.6	0.011	0.013
8.0	0.028	0.033
8.4	0.069	0.079
8.8	0.152	0.178
9.2	0.319	0.358

It negatively influences growth, feeding, survival, susceptibility to diseases and parasites. When ammonia levels get higher, it will become difficult to extract energy from feed. This makes the culture animals lethargic and ultimately leads to coma and death. Toxicity of ammonia decreases with the increasing Dissolved Oxygen concentration. The concentration of ammonia decreases with the increasing concentration of carbon dioxide, decreasing pH, increasing Calcium ions. An excess of ammonia may lead to adverse physiological consequences and mortalities.

Metabolic ammonia produced inside the body of the culture animals enters the blood and is transported to the gills for excretion into the surrounding water. If water, on the other hand, has more concentration of ammonia than that of the blood, then the ammonia, instead of being excreted into the water, remains in the blood itself. This causes physiological negative effects on the retention for a long time in the blood. Ammonia beyond the safety level reduces growth, increases Oxygen consumption, and alters concentration of hemolymph proteins and free amino acid levels. It may cause high mortalities.

Mitigation of Ammonia Problems:

Naturally the released or already existing ammonia in the pond water decreases by the uptake of plankton, oxidation of the same into less toxic Nitrite and Nitrate forms by nitrifying bacteria like nitrobacter and nitrosomonas.

1. Reduction of feeding
2. Liming – hydrated or quick lime
3. Fertilizing the pond – Algae grow and consume ammonia
4. Increasing the depth of the pond – volume of water increases and concentration goes down
5. Aeration – High quality aeration helps
6. Water exchange – 10-50% water helps reduce ammonia concentration
7. Adding well water
8. Addition of organic carbon source
9. Addition of acid
10. Addition of bacterial amendments
11. Application of ion-exchange chemicals

OBJECTIVES OF THE STUDY

- To assess the Total Ammonia Nitrogen of the three ponds with different stocking densities
- To evaluate the unionized toxic ammonia concentration of the three ponds with different stocking densities

Hypothesis:

1. There is no significant difference among the ponds of various stocking densities with respect to TAN values
2. There is no significant difference among the ponds of various stocking densities with respect to UIA values

REVIEW LITERATURE

Shrimp farming plays a pivotal role in the socio-economic condition of the coastal population of India by way of contributing to foreign exchange earnings and livelihood options. The farmers are going for gradual intensification with more than the CAA prescribed Stocking Densities. Optimum stocking density at which sustainable growth is achieved is to be investigated. The optimum density varies with the culture water. The present study carried out in fresh water tried to assess the optimum stocking density for the fresh water shrimp culture.

Most of the researchers conducted research on shrimp economic performance at various stocking densities. They are Allan. G.L. and G.B. Maguire et al., (1992), Daniels, W.H., D'Abramo, L.R. Fonden., Durant.M.D.(1995), Apud F.D., K. Gonzalez and N. Deatras (1981), Maguire, G. B. and M. I. Leedow.(1983), Tidwell.J.H. Coyle, S., Weibel, C. and Evans, J.(1999) Whay-Ming, R. and C. Yew-Hu.(1992). Hargreaves & Tucuker (2004) conducted studies on the harmful effects of ammonia on culture animals. Van Wyk et al, (1999) inferred on the LC50 values of ammonia in *L.vannamei.* Qun Liu et al., (2016) conducted similar experiments on Rain-bow trout, *Onchorynchus mykiss.*

METHODOLOGY OF THE STUDY

Study was conducted in the chosen three ponds in the village, Epurupalem, Chirala area of coastal Andhra Pradesh. The samples were collected 5 times fortnightly beginning the first sample on 15th November, 2019 and with the last sample on 15th January, 2020. All the samples were analyzed in Anu Lab, a network lab in coastal area of the state.

TAN remains odorless and colorless in the shrimp culture ponds. Therefore laboratory methods are only option for checking the ammonia in the ponds. TAN is measured according to UNESCO (1983). One Way ANOVA (Zar, 1996) was employed to see if there is significant difference between the two values of ammonia measured from the two culture ponds under study. Difference is considered significant at 95%.

Un-ionized ammonia concentration (NH_3) = TAN x Multiplication Factor

RESULTS AND DISCUSSION

Table.3: Average TAN & UIA values

Stocking Density	T50	T70	T90
TAN(mg/L)	0.10	0.25	0.50
UIA(mg/L)	0.005	0.009	0.024

Fig. 3 **Graph showing the trend in the TAN & UIA values in three ponds of different Stocking densities**

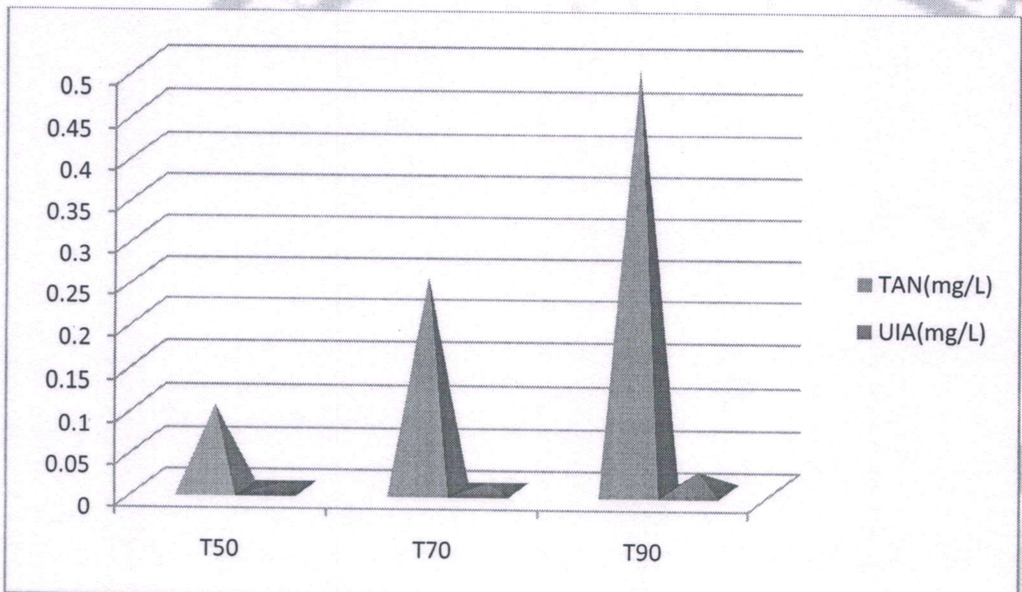


Table 5.0: ANOVA Result of three ponds with respect to ammonia

Source	Bracket Term	SS	df	MS	f	p value
Between Groups (A)	(A) = 0.002579	0.000803	2	0.0004015	1.38	0.4892
Within Groups (Y)	(Y) = 0.00520	0.002621	9	0.0002912		
Total	(T) = 0.001776					

The results show that the toxic un-ionized form of ammonia increases with the increasing stocking density. As the stocking density increases, the feed input increases, the ammonia excreted also goes up in the pond. The management measures with respect to this form of ammonia will also get affected. This may be the reason why the ammonia value is showing an upward trend with the increasing stocking density. The results of this study are in line with the ammonia values obtained in the culture of Rainbow Trout (*Onchorynchus mykiss)* where the pond with SD3 showed highest values of un-ionized ammonia (Qun Liu et al., 2016). Lethal concentration of ammonia for *L.vannamei* is 2.78 ppm.

The present study shows the values well below the lethal and sub-lethal levels of ammonia in the ponds of shrimp culture studied. Maintainance of good water quality is essential for the success of aqua-farming (Devi, 2013). One of the frontline parameter to be kept under check is the most toxic form of ammonia i.e. un-ionized form of ammonia. Usually TAN is controlled by the addition of white sugar to the pond. The F statistic calculated for the data (1.38) is much lower than the table value (4.5) at 0.05 level of significance (p alpha value = 0.05). The calculated p value is 0.4892. Since the F value is only slightly larger than the normal 1.0 and the p value is larger than 0.05 there is weak evidence against the null hypothesis. This suggests that there is not a significant difference among the three ponds of different stocking densities with respect to the ammonia levels.

Since 1994 భారతదేశపు మొట్టమొదటి అధునాతన ఆక్వా ల్యాబొరేటరి

ANU LABS AQUA DOCTOR

అనూ ల్యాబ్స్

Reg. No.

PCR LAB

WATER ANALYSIS REPORT

నీటి పరీక్ష విశ్లేషణ

నీటి ప్రమాణాలు		Optimum Levels PRAWN	FISH	7AP	
pH	పిహెచ్	7.5 to 8.5	7.5 to 8.5	7.98	
Salinity (ppt)	సెలినిటీ	5 - 25 ppt	0 ppt	5	
CO_3 (ppm)	కార్బొనేట్లు	20 - 40 ppm	20 - 40 ppm	Nil	
HCO_3 (ppm)	బైకార్బొనేట్లు	100 - 150 ppm	150 - 500 ppm	90	
T. Alkalinity (ppm)	మొత్తం ఆల్కలినిటీ	150 - 250 ppm	150 - 500 ppm	90	
T. Hardness	మొత్తం కఠినత	సెలినిటీ మీద ఆధారపడుతుంది		1560	
Mg (ppm)	మెగ్నీషియం	సెలినిటీ మీద ఆధారపడుతుంది		194	
Ca (ppm)	కాల్షియం	సెలినిటీ మీద ఆధారపడుతుంది		132	
Potassium (ppm)	పొటాషియం	సెలినిటీ మీద ఆధారపడుతుంది		-	
Sodium (ppm)	సోడియం	సెలినిటీ మీద ఆధారపడుతుంది		-	
T. Ammonia (ppm)	మొత్తం అమోనియా	0.4 - 2 ppm	0.4 - 2 ppm	0.1	
U. Ammonia (ppm)	అయనీకరణ జరిగిన అమోనియా	0 - 0.01 ppm	0 - 0.01 ppm	0.005	
Residual Chlorine	అవశేష క్లోరిన్	0 - 0.2 ppm	0 - 0.4 ppm	-	
Others				-	
NO_2 (ppm)	నైట్రేట్	సెలినిటీ మీద ఆధారపడుతుంది		Nil	
Iron (ppm)	ఇనుము	0 - 0.1 ppm	0 - 0.1 ppm	-	
HS (ppm)	హైడ్రోజన్ సల్ఫైడ్	0 - 0.4 ppm	0 - 0.4 ppm	-	
D.O (ppm)	ఆక్సిజన్	4 - 10 ppm	2 - 10 ppm	4.61	
Electrical Conductivity(EC) us/cm		> 1.7	> 1.7	-	
Redox Potential : mV		< - 100	< - 100	-	
[illegible]		25C - 35C	25C - 35C	31.4°C	

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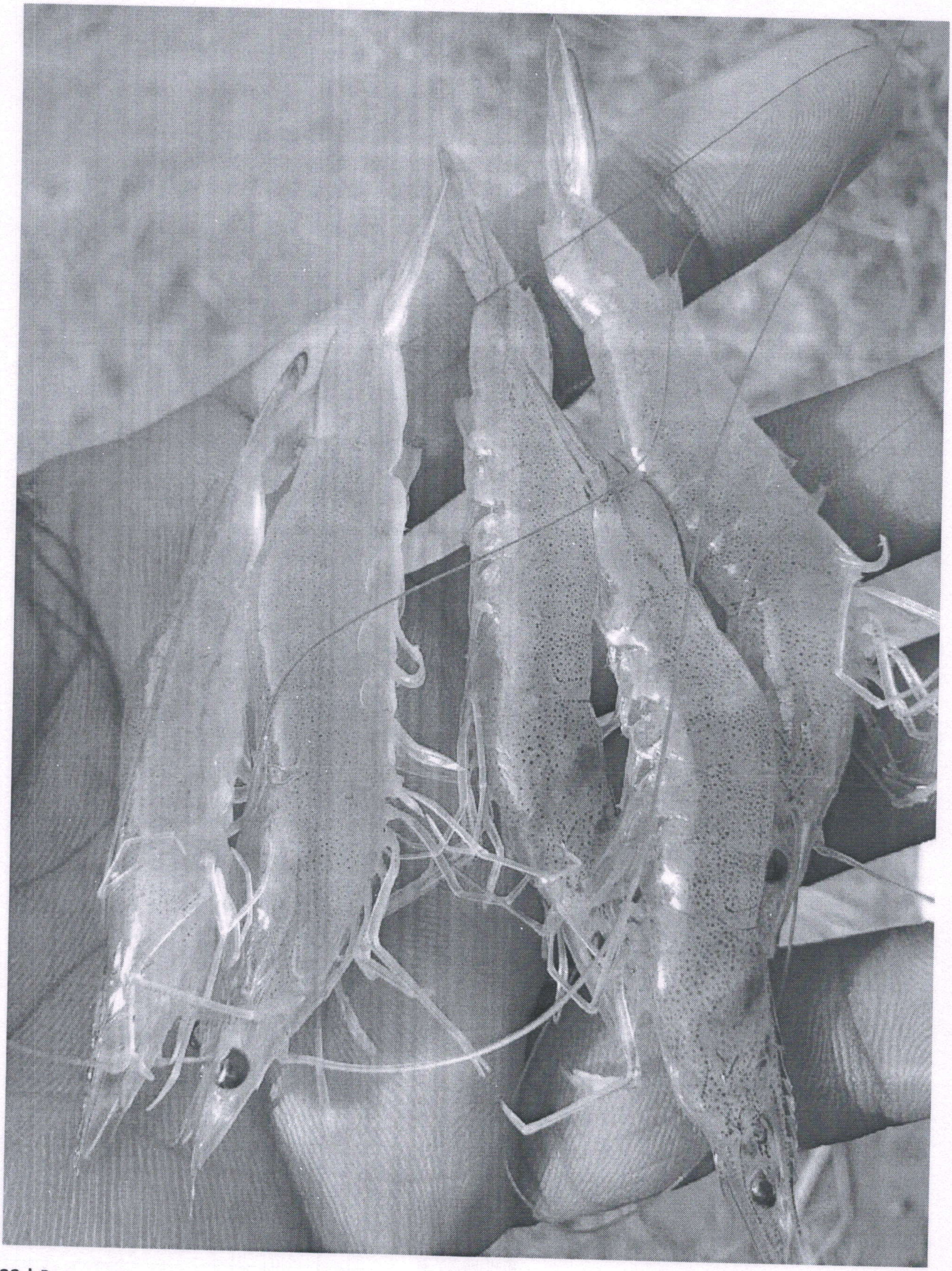
నీటి ప్రమాణాలు		Optimum Levels PRAWN	Optimum Levels FISH	10A	
pH	పిహెచ్	7.5 to 8.5	7.5 to 8.5	8.38	
Salinity (ppt)	సెలినిటీ	5 - 25 ppt	0 ppt	2.5	
CO_3(ppm)	కార్బోనేట్లు	20 - 40 ppm	20 - 40 ppm	12	
HCO_3(ppm)	బైకార్బోనేట్లు	100 - 150 ppm	150 - 500 ppm	168	
T.Alkalinity (ppm)	మొత్తం ఆల్కలినిటీ	150 - 250 ppm	150 - 500 ppm	180	
T.Hardness	మొత్తం కఠినత	సెలినిటీ మీద ఆధారపడుతుంది		1100	
Mg (ppm)	మెగ్నీషియం	సెలినిటీ మీద ఆధారపడుతుంది		135	
Ca (ppm)	కాల్షియం	సెలినిటీ మీద ఆధారపడుతుంది		216	
Potassium (ppm)	పొటాషియం	సెలినిటీ మీద ఆధారపడుతుంది		–	
Sodium (ppm)	సోడియం	సెలినిటీ మీద ఆధారపడుతుంది		–	
T.Ammonia (ppm)	మొత్తం అమోనియా	0.4 - 2 ppm	0.4 - 2 ppm	0.80	
U.Ammonia (ppm)	అయనీకరణ జరగని అమోనియా	0 - 0.01 ppm	0 - 0.01 ppm	–	
Residual Chlorine	అవశేష క్లోరిన్	0 - 0.2 ppm	0 - 0.4 ppm	0.0	
Others				–	
NO_2(ppm)	నైట్రేట్	సెలినిటీ మీద ఆధారపడుతుంది		0.06	
Iron (ppm)	ఇనుము	0 - 0.1 ppm	0 - 0.1 ppm	–	
H_2S (ppm)	హైడ్రోజన్ సల్ఫైడ్	0 - 0.4 ppm	0 - 0.4 ppm	–	
D.O (ppm)	ఆక్సిజన్	4 - 10 ppm	2 - 10 ppm	5.02	
Electrical Conductivity(EC) us/cm		> 1.7	> 1.7	1.1	
Redox Potential : mV		< - 100	< - 100	102.8	
Water Temp.	నీటి ఉష్ణోగ్రత	25C - 35C	25C - 35C	26.8	

Suggestions :



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Reg. No.

WATER ANALYSIS REPORT

నీటి పరీక్ష విశ్లేషణ

Days of C

నీటి ప్రమాణాలు		Optimum Levels PRAWN	FISH	10 AC	
pH	[illegible]	7.5 to 8.5	7.5 to 8.5	7.66	
Salinity (ppt)	[illegible]	5 - 25 ppt	0 ppt	9	
CO_2 (ppm)	[illegible]	20 - 40 ppm	20 - 40 ppm	nil	
HCO_3 (ppm)	[illegible]	100 - 150 ppm	150 - 500 ppm	112	
T. Alkalinity (ppm)	[illegible]	150 - 250 ppm	150 - 500 ppm	113	
T. Hardness	[illegible]	[illegible]		2880	
Mg (ppm)	[illegible]	[illegible]		441	
Ca (ppm)	[illegible]	[illegible]		384	
Potassium (ppm)	[illegible]	[illegible]		-	
Sodium (ppm)	[illegible]	[illegible]			
T. Ammonia (ppm)	[illegible]	0.4 - 2 ppm	0.4 - 2 ppm	0.15	
U. Ammonia (ppm)	[illegible]	0 - 0.01 ppm	0 - 0.01 ppm	0.009	
Residual Chorine	[illegible]	0 - 0.2 ppm	0 - 0.4 ppm	-	
Others					
NO_2 (ppm)	[illegible]	[illegible]		nil	
Iron (ppm)	[illegible]	0 - 0.1 ppm	0 - 0.1 ppm	-	
H_2S (ppm)	[illegible]	0 - 0.4 ppm	0 - 0.4 ppm	-	
D.O (ppm)	[illegible]	4 - 10 ppm	2 - 10 ppm	4.88	
Electrical Conductivity(EC) us/cm		> 1.7	> 1.7		
Redox Potential mV		< -100	< -100		
Water Temp.	[illegible]	25C - 35C	25C - 35C	24.8c	
Suggestions					

SUMMARY AND CONCLUSION

The shrimp cultured at various stocking densities usually show difference with respect to the water quality parameters. In the present study, the ammonia levels show little variation among the ponds of various stocking densities. Hence, the higher intensive densities may show significant difference with respect to the ammonia levels.

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