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Swollen hind gut disease in postlarvae of *Penaeus monodon*: Aetiological studies

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Abstract
Prevalence of Swollen Hind Gut (SHG) of shrimp post larvae produced from the hatcheries located along Kakinada and Visakhapatnam coasts of Andhra Pradesh were 12.50 and 17.85 % respectively during the period 2012-'13. Samples of post larvae and water and water from the SHG affected tanks were subjected to water quality, microbiological and histopathological analysis. *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* were isolated from the SHG affected individuals. High positive correlation (r>0.55) was observed between pH & Temperature and Nitrate & Temperature of water in larval rearing tanks. T-test revealed that there was highly significant difference between Temperature, pH, Dissolved Oxygen, Nitrates, Ammonia, Phosphates, Silicates, Calcium and Magnesium. Histopathological studies revealed degeneration and vacuolization of the Hepatopancreatic tubules. Muscle layers in the gut showed excessive growth leading to the reduced lumen with swollen caecae on the distal end of the midgut region.

Keywords: *Penaeus monodon*, postlarvae, Swollen Hind Gut, Shrimp hatcheries, water quality, histopathology

1. Introduction
Culture of tiger shrimp, *Penaeus monodon*, was one of the most profitable venture in aquaculture sector in India and in many countries of the world but recently, culture of *Litopenaeus vannamei* has taken its place, since its inception in 2008 to India, contributing a major share in the Indian shrimp export. Seed for stocking in culture ponds is mostly procured from commercial shrimp hatcheries as the availability and quality of wild seeds varies. Optimum qualities of early (egg and nauplius) or late (postlarva) stages are of primary importance to obtain maximum yields during larval and postlarval culture as well as for further growout [1]. Losses of shrimp larval production in hatcheries due to luminous vibriosis were found to be catastrophic and could not be controlled even after the use of antibiotics [2]. Similarly, the post larvae were also affected by several diseases like MBV, WSV, multiple viral infections, filamentous bacterial infections and larval mycosis [3-10]. Mass mortality of larval stages have also been increasingly observed in many hatcheries but the cause of these problems remains poorly understood.
The understanding of the water quality parameters and in shrimp hatcheries and culture ponds can help us understand and solve some of the diseases and issues faced by shrimp farmers [11]. The monitoring of water quality parameters allows the control and even predicts the occurrence of unfavourable conditions for cultivation, thus avoiding risks of environmental damage and breakage of the production process [12]. Newly emerging diseases in shrimp hatcheries have always been challenging to the shrimp farmers as well as to the shrimp pathologists. One such new problem is Swollen Hind Gut syndrome (SHG). Swollen Hind Gut syndrome (SHG) was first reported by Lavilla-Pitago *et al.*, (2002) [13] in post larvae of *Penaeus monodon* and defined it as a morphological deformity that tends to occur during late post larval (PL) stage, typically after PL10. Swollen hindgut syndrome (SHG) is an emerging problem in the shrimp hatcheries along the East coast of India. Shrimp larvae with SHG show a swollen hindgut with or without melanisation and this condition is also referred to as hindgut expansion and the shrimp farmers avoid stocking them. Rhythmic movements in the rectal region of the affected larvae result in difficulty of expelling the faecal pellets [14, 15] but exhibit normal swimming behaviour.

Presently, the occurrence of low survival rates, size variation, occurrence of white faecal strands and loose shell syndrome in grow out ponds are attributed to the presence of SHG in seeds. Hence, the present study focusses on water quality parameters in control and SHG affected larval rearing tanks to establish correlation besides microbiological and histopathological studies.

2. Materials and Methods

2.1 Survey

A general survey was undertaken during the period 2012-13 on shrimp hatcheries located along the coasts of Visakhapatnam and Kakinada (12 and 25 hatcheries respectively), Andhra Pradesh, India (Fig. 1) to find out the prevalence of SHG through a questionnaire.

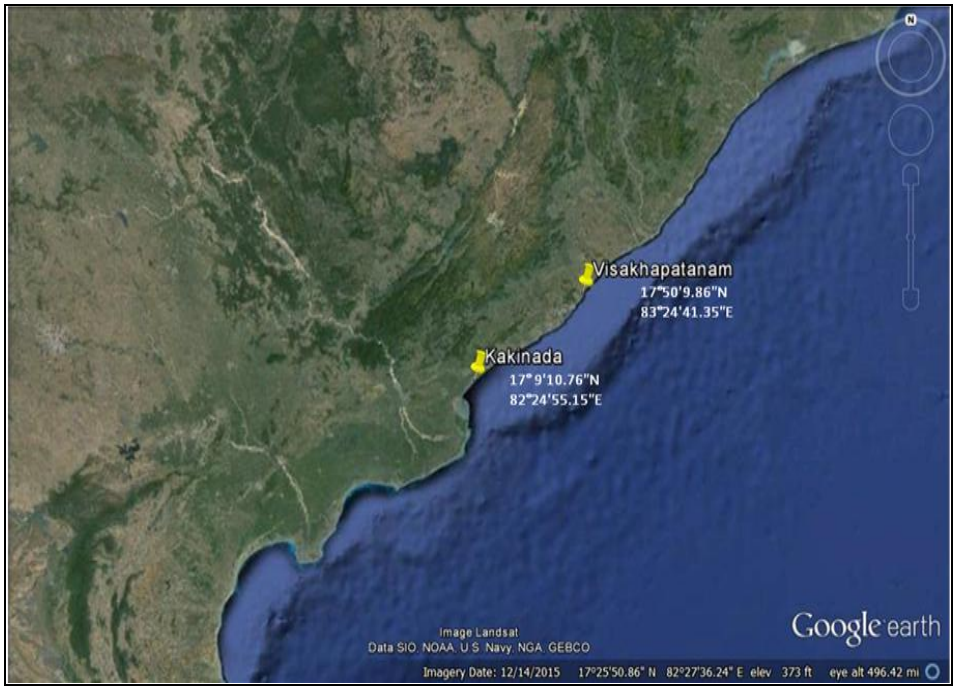


Fig 1: Map showing the study area.

2.2 Sample Collection

Water samples were collected for 5 cycles (each cycle consisted of 5 batches of post larvae reared in 5 different tanks) from a commercial shrimp hatchery located in Visakhapatnam. Different water quality parameters (physico-chemical and microbiological) were analyzed following standard methods [16]. The average values of each parameter from all the five tanks in each cycle were tabulated and taken as standard to compare with the SHG affected tanks. The photograph showing normal hind gut region in shown in Fig. 2. The Postlarvae (Pl's) affected with Swollen Hind Gut (SHG) (Fig. 3) were collected and brought to the laboratory and subjected to water quality, microbiological and histopathological analysis.

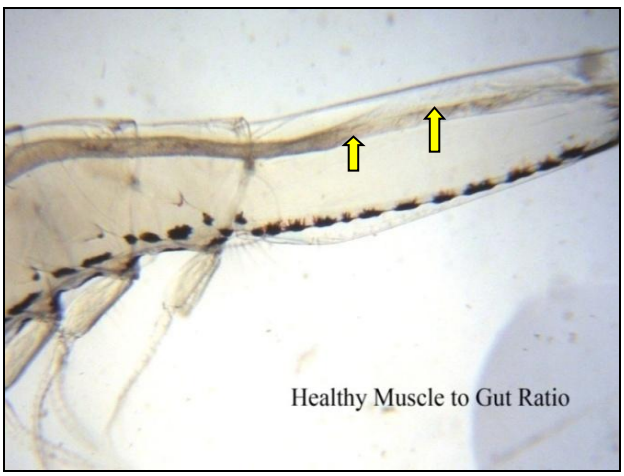


Fig. 2: Photograph showing normal Hindgut region

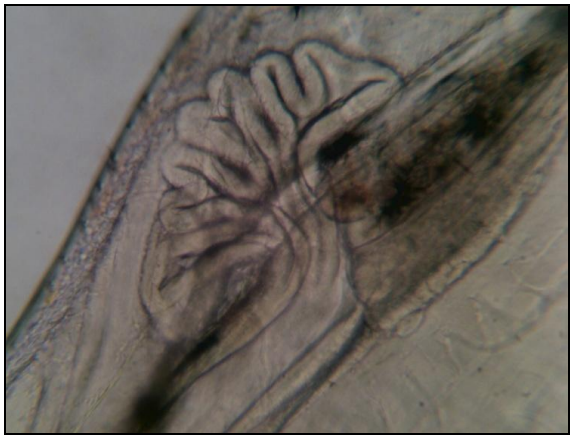


Fig. 3: Photograph showing enlarged view of the Swollen Hind Gut with foldings (400 X) (Severe Infection)

2.3 Water Quality Analysis

Water Quality parameters such as Salinity, Temperature, pH, Alkalinity, Dissolved Oxygen (D.O), Nitrates, Nitrites, Ammonia, Phosphates, Silicates and Calcium and Total Hardness were measured by following standard methods [16].

2.4 Statistical Analysis

The data obtained on water quality parameters was analyzed for T- test and Correlation by using SPSS software (Version 17.0).

2.5 Microbiological Studies

SHG affected post larvae were brought to the laboratory in live condition washed thoroughly with sterile distilled water and homogenized well in a tissue homogenizer with 0.85 %

Normal Saline. The homogenized sample mixture thus obtained was serially diluted and pour plated onto Zobell's Marine Agar (ZMA) and Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS). Similarly water from the affected hatchery tanks was also pour plated on ZMA and TCBS. All the plates were incubated for 18-24 hrs at 27^0C. Upon incubation those colonies which appeared dominant and distinct were counted and selected for taxonomical studies [17]. Virulence studies were carried out in order to satisfy Koch's postulates by bath challenging the Post larvae with concentration of 3 x 10^7 CFU / g to healthy post larvae following Liu *et al*., (1996) [18]. Two bacterial isolates which showed maximum virulence out of 56 were selected and designated as SHG 1 and SHG 2.

2.6 Antibiotic sensitivity tests

Two isolates SHG 1 and SHG 2 were separately spread over MHA dishes and various antibiotic discs (Himedia, Bangalore) were placed over them carefully (4 discs per dish) and incubated for 18-24 hrs at 32^0C following Bauer *et al*., (1966) [19]. Upon incubation, the inhibition zones obtained around the discs were measured.

2.7 Histopathological studies

SHG affected shrimp larvae were fixed in Davidson's fluid (DF), processed and stained following the method given by Bell and Lightner (1988) [20].

3. Results

3.1 Survey

The prevalence of SHG was lower in Kakinada coast (12.50%) than in that of Visakhapatnam coast (17.85 %) (Table No.1).

3.2 Water Quality Analysis

The selected hatchery was found to be maintaining good management practices (GMP) as there was no incidence of any disease outbreak during our investigation period. Average values of 5 batches in each cycle were tabulated and designated as standard tables to compare with the disease affected ones. Data on water quality parameters of normal post larval tanks were represented graphically (Figs. 4-15) and data on SHG affected post larval tanks were tabulated in Table No. 2.

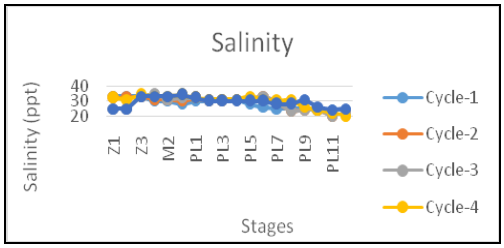
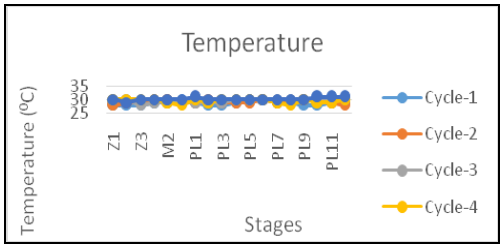


Fig 4



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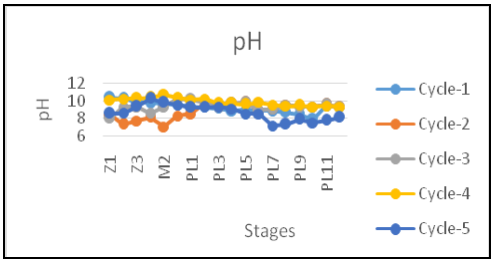

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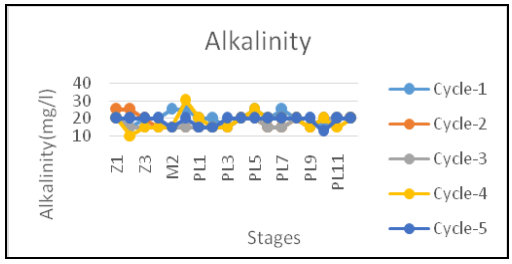

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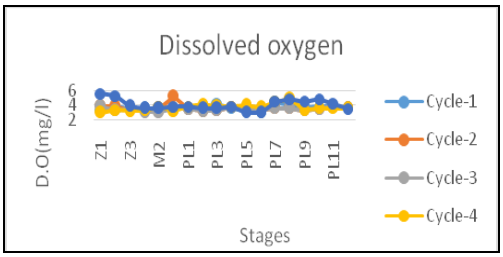

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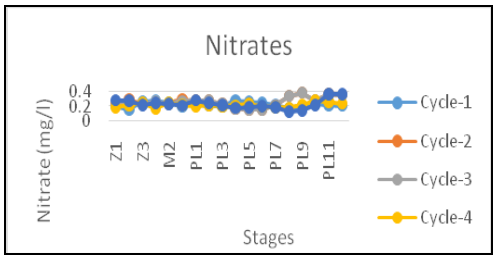

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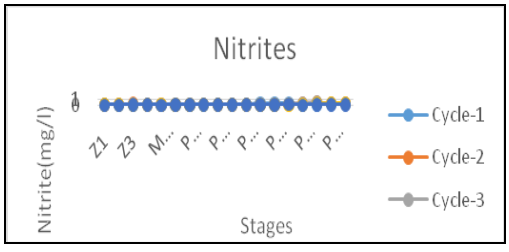

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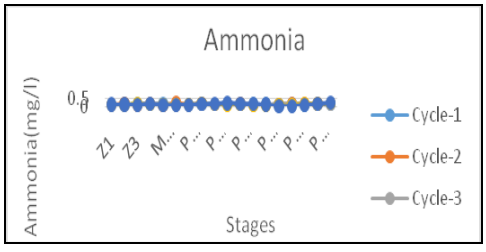

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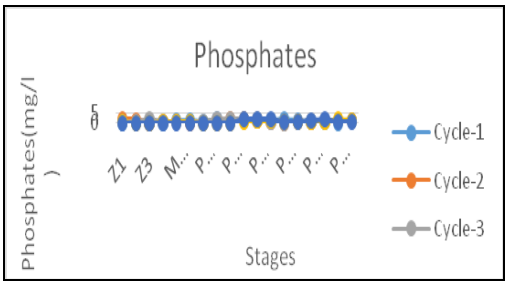

Fig.12

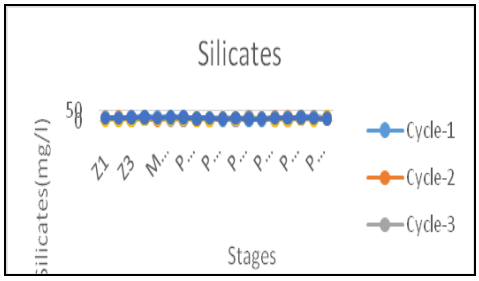

Fig 13

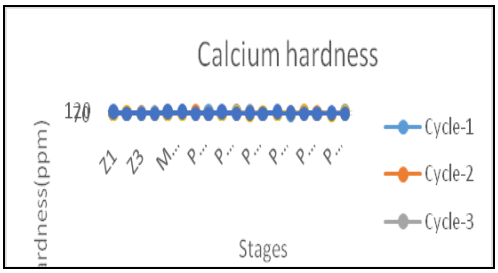

Fig 14

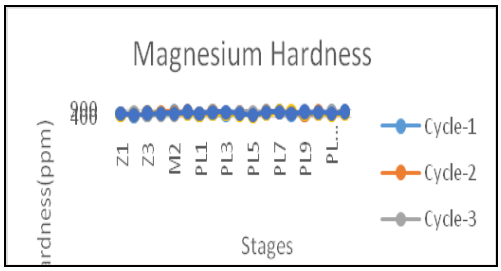

Fig 15

Figures 4-15: Water quality parameters in a larval rearing tanks of a standard hatchery

Table 1: Summary of the data collected through Questionnaire

Parameter	Observations	
Survey areas	**Hatcheries in Kakinada coast**	**Hatcheries in Visakhapatnam coast**
Type of Hatchery	100 % Coastal	100 % Coastal
Culture Species Produced	43 % *P. monodon*	57 % *L. vannamei*
Culture Technology	55 % Galveston 45 % Green Water	13.57 % Galveston 82.86 % Green Water 3.57 % Mixed
Brooder Source	Local	Exotic
Brooder Avg. Wt. (g)	175 g	45 g
Type of Spawning	Natural Spawning	Induced Breeding (Eye stalk ablation) : 89.28 % Direct nauplius Stocking : 10.72 %
Hatcheries carrying out tests for Brooders (WSSV , MBV)	77.50 %	61.43 %
Fecundity	~ 8 lakh eggs / brooder / spawn	~ 1.25 lakh eggs / brooder / spawn
Average Survival Rate	60 %	42.5 %
Commonly Occurring Diseases	(Out of 100 %)	
Conversion problems (%)	25.00	40.71
Overmoulting (%)	38.75	40.00
Swollen hind gut (%)	12.50	17.85
Luminescent bacteria (%)	32.50	
Zoeal syndrome (%)	--	65.00
Vibriosis (%)	--	3.57
Necrosis (%)	30.00	--
Feed		
Zoea & Mysis	Chaetoceros + Micro encapsulated feeds	Chaetoceros + Micro encapsulated feeds
Post Larvae	Artemia live and flakes + Micro encapsulated feeds	Artemia live and flakes + Micro encapsulated feeds
Probiotic Usage		
Feed Probiotic	30.00 %	--
Water Probiotic	57.50 %	89.29 %
Feed and Water Probiotic	--	7.14 %
Testing of Basic Water Quality Parameters like Temperature, pH, D.O , Salinity, Alkalinity	56.25 %	29.28 %
Other Water Quality parameters like Nitrites, Nitrates, Silicates, Ammonia, Phosphates, Calcium, Magnesium	NOT BEING TESTED	NOT BEING TESTED
Bacteriological testing	18.75 %	24.28 %
Sea Ranching	45.00 % of total hatcheries	37.85 % of total hatcheries

Table 2: Water quality parameters of the diseased (SHG) post larvae

Stage	Salinity (ppt)	Temper-ature	pH	Alkalinity (mg/L) as$CaCO_3$	D.O. (mg/l	Nitrate (mg/L)	Nitrite (µg/l)	Ammonia (mg/l)	Phosphates (mg/l)	Silicates (mg/l)	Calcium Hardness (ppm)	Magnesium Hardness (ppm)
PL4	29	30	9.09	15	3.8	0.18	0.06	0.19	1.91	12.4	95	650
PL5	28	30	8.54	15	3.5	0.19	0.08	0.15	2.05	15.5	90	600
PL6	28	30	8.59	20	3.5	0.2	0.11	0.14	1.8	12	95	550
PL7	25	30	8.54	15	4.5	0.19	0.11	0.12	0.49	11.8	85	550
PL8	25	30	8.74	20	4.8	0.13	0.15	0.02	0.52	15.47	80	500
PL9	25	30	8.6	20	4.5	0.15	0.05	0.02	1.43	16.58	95	650
PL10	24	31	9.24	15	4.8	0.22	0.09	0.08	2.05	17.7	85	600
PL11	21	31	9.26	15	4.2	0.36	0.07	0.17	0.66	15.45	80	650
PL12	21	31	9.24	20	3.5	0.36	0.06	0.22	0.58	13.61	95	700

3.3 Statistical Analysis

The summary of the Correlation obtained between various water quality parameters of normal and SHG affected larval tanks were presented in Table No : 3.

High positive correlation (r= >0.55) was observed between pH and Salinity, Alkalinity and Temperature, nitrate and nitrite in majority of cycles. High positive correlation was also found between Ammonia and D.O, D.O and alkalinity, silicates and temperature, magnesium and temperature, magnesium and D.O, Phosphates and Nitrites only in lesser number of cycles. High negative correlation (r=<0.55) was found between nitrite and salinity, silicate and salinity in majority of the cycles. High negative correlation was also found between temperature and salinity, alkalinity and salinity, Silicate and pH, nitrite and pH only in lesser number of cycles. Significant positive correlation (r=<0.54) was observed between phosphate and alkalinity, magnesium and alkalinity, silicates and alkalinity, magnesium and calcium and ammonia and nitrate in majority of the cycles. Significant positive correlation was also found between ammonia and alkalinity, phosphates and temperature, magnesium and silicates and nitrite and nitrate in lesser number of cycles. Significant negative correlation (r=<0) was observed between pH and salinity, calcium and alkalinity, phosphates and salinity, phosphates and pH, calcium and phosphate & D.O and salinity.

However, in case of SHG affected tanks high positive correlation (r>0.55) was obtained between pH & temperature and nitrate & temperature. Similarly high negative correlation (r=<-0.55) was obtained between temperature & salinity, and magnesium & nitrite. Positive correlation (r=>0.1 to <0.54) was obtained between nitrate & pH, ammonia & nitrate and phosphate & salinity. However, there was no significant negative correlation between any other parameters in the disease affected tanks.

Summary of the T-test

Statistical analysis comparing the water quality parameters of healthy and SHG affected tanks revealed that there was highly significant difference between Temperature, pH and significant difference between salinity, alkalinity, Dissolved Oxygen and Magnesium when compared with the SHG affected tanks (Table No : 4).

Table 3: Summary of the correlation coefficient (r) matrix between various water quality parameters tested

	High Positive correlation (r=>0.55)	High Negative correlation (r=<-0.55)	Significant Positivecorrelation (r=>0,<0.54)	SignificantNegative correlation (r=<0)
Cycle I	pH & Salinity Alkalinity & Temperature	Nitrite & Salinity	Ammonia & Alkalinity Phosphates & Temperature Phosphates & Alkalinity Magnesium & Alkalinity Magnesium & Silicates	–
Cycle II	Ammonia & Dissolved Oxygen	Nitrate &Salinity Nitrite & Salinity Silicates & Salinity	Nitrite & Nitrate Phosphates & Alkalinity Silicates & Alkalinity	pH & Salinity Calcium & Alkalinity
Cycle III	Alkalinity & Temperature Dissolved Oxygen & Alkalinity Nitrite & Nitrate Silicates & Temperature Magnesium & Temperature Magnesium & Dissolved Oxygen	Temperature & Salinity Alkalinity & Salinity Nitrate & Salinity Nitrite & Salinity Silicates & Salinity Silicates & pH	Silicates & Alkalinity Magnesium & Alkalinity Magnesium & Calcium	–
Cycle IV	pH & Salinity Nitrite & Nitrate Phosphates & Nitrite	Nitrite & Salinity Nitrite & pH	Ammonia & Nitrate Ammonia & Nitrite Magnesium & Calcium	Phosphates & Salinity Phosphates & pH Calcium & Phosphates
Cycle V	pH & Salinity	-	-	Dissolved Oxygen & Salinity
SHG affected Tanks	pH & Temperature Nitrate & Temperature	Temperature & Salinity Magnesium & Nitrite	Nitrate & pH Ammonia & Nitrate Phosphates & Salinity	-

Table 4: t- values of different water quality parameters in healthy and SHG affected tanks

Stages	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Salinity	0.538@	0.593@	0.593@	1.336@	2.267*
Temperature	4.619**	4.000**	2.000@	3.024**	0.000@
pH	0.325@	3.549**	3.504**	4.993**	3.755**
Alkalinity	2.502*	0.918@	0.918@	1.668@	1.606@
DO	1.825@	2.816*	2.816*	0.897@	0.367@
Nitrate	0.300@	1.039@	1.039@	0.148@	0.000@
Nitrite	2.811*	1.854@	1.854@	1.034@	0.074@
Ammonia	0.906@	0.179@	0.179@	1.156@	0.000@
Phosphates	0.343@	0.861@	0.861@	1.007@	0.034@
Silicates	1.043@	1.707@	1.707@	0.526@	0.000@
Calcium	1.073@	1.015@	1.990@	1.026@	0.597@
Magnesium	0.724@	1.315@	3.232**	0.351@	2.545*

3.4 Microbiological Studies

On the basis of standard taxonomical keys the isolates SHG 1 and SHG 2 have been identified as *V. parahaemolyticus* and *Pseudomonas aeruginosa* respectively (Table No : 5).

Table 5: Morphological and biochemical characteristics of bacterial isolates from SHG affected shrimp PL's

Name of the test	Isolate 1 (*V. para*)	Isolate 2 (*P. aeru*)
Gram's staining	-	-
Shape	Rod	Rod
Motility	+	+
Oxidase	+	+
Catalase	+	+
O/F test	F	O
Acid production from glucose	+	+
NaCl tolerance test		
2%	+	+
4%	+	+
6%	+	+
8%	+	+
10%	+	+
Temperature tolerance test		
4 ^{0}C	-	-
20 ^{0}C	+	+
30 ^{0}C	+	+
40 ^{0}C	+	+
Decarboxylation of amino acids		
Arginine	-	+
Ornithine	+	-
Lysine	+	-
MRVP test	+	+
VP test	-	-
Indole test	+	+
Starch hydrolysis	+	-
Urea hydrolysis	+	+
Esculin hydrolysis	-	-
Gelatin liquefaction	+	+
Utilisation of carbohydrates		
L-Arabinose	-	+
Dextrose	+	+
Fructose	+	+
Lactose	-	-
Mannose	+	-
Galactose	+	+
Sucrose	-	-
Trehalose	+	+
Cellobiose	-	-
Melibiose	-	-
Salicin	-	-
Xylose	-	+
Citrate utilisation	+	+
Nitrate reduction	+	+
ONPG hydrolysis	-	-
Growth on TCBS	Y	Y
Inhibition by 0/129 phosphate		
10 µg	R	R
150 µg	S	S
Luminescence	-	-

V. para. = Vibrio parahaemolyticus, P. aeru. = Pseudomonas aeruginosa

3.5 Antibiotic Sensitivity Tests

Both the isolates (SHG 1 and SHG 2) were highly sensitive to ciprofloxacin and gentamycin but resistant to norfloxacin, ampicillin and penicillin G. SHG 1 is sensitive to erythromycin and SHG 2 is sensitive to tetracycline. In addition, SHG 1 showed resistance to oxytetracycline and streptomycin (Table No. 6)

Table 6: Antibiotic Sensitivity results of bacterial isolates of SHG

Name of the Antibiotic	Diameter of Zone of inhibition (mm)	
	SHG 1	SHG 2
Norfloxacin	R	R
Ciprofloxacin	27	18
Oxytetracycline	R	13
Chloramphenicol	10	14
Gentamycin	28	20
Ampicillin	R	R
Kanamycin	17	19
Penicillin G	R	R
Tetracycline	14	23
Furazolidone	14	13
Erythromycin	20	13
Streptomycin	R	16

R = Resistant

3.6 Histopathological studies (SHG)

Pronounced changes were observed in the histological sections especially in the Hepatopancreas and gut of the post larvae affected by SHG. Degeneration and vacuolization of the HP tubules were noticed (Fig. 16) of post larvae affected by SHG. Besides the excessive growth at the distal end of the mid gut region with swollen caecae (Fig. No's : 17 & 18).

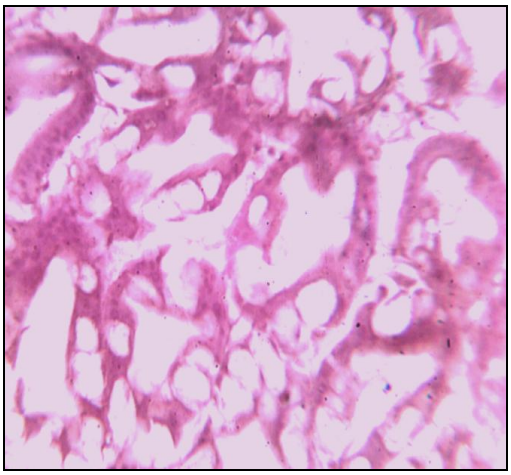

Fig 16: Section of HP showing vacuolization and disintegration of cells in the HP tubules (400X)

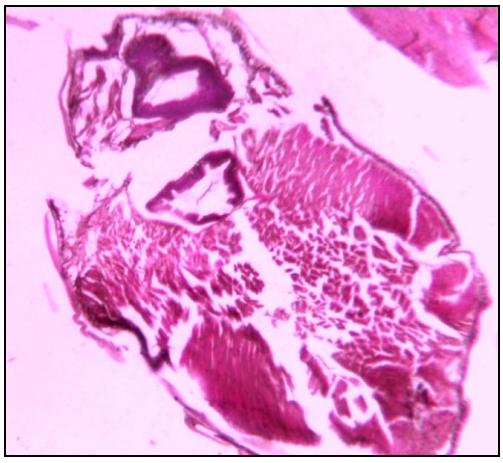

Fig 17: T.S. of Pl caecae showing the excessive growth of muscle layers (100 X)

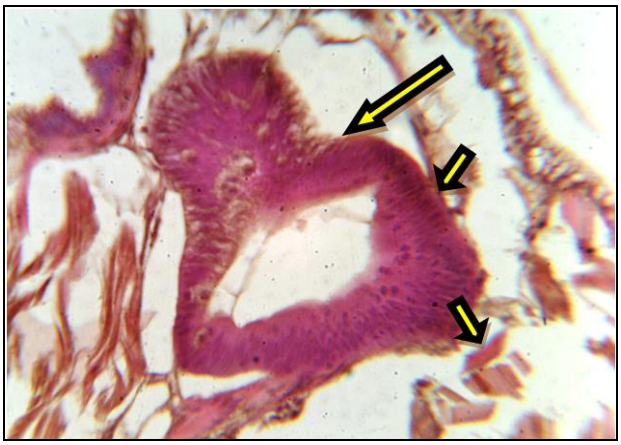

Fig 18: T.S. of Pl caecae showing the excessive growth of muscle layers, leading to the reduced lumen (400X)

4. Discussion

Survey revealed that 12.50 % and 17.85 % of the shrimp hatcheries have been facing the problem of Swollen Hind Gut (SHG) in Kakinada and Visakhapatnam coasts respectively. The role of physico-chemical parameters such as salinity and water temperature in relation to survival, growth and production of *P. monodon* was studied earlier [21-23]. The optimal pH for the normal growth of marine shrimp species is 6 to 9 [24]. pH values ranged between 7.05 and 10.73 in the larval rearing tanks of the shrimp hatcheries studied. Post-larvae (Pl) can withstand the salinity changes between 10-20 ppt. [25]. The highest and the lowest salinity levels recorded in the present study varied from 28 – 31 ppt. Alkalinity is related to important factors in shrimp culture: buffer effect on daily variation of pH in the pond, setting the soluble iron precipitated and particularly in ecdysis (molting) and growth [26]. Low alkalinity gives low buffering capacity and leads to high pH fluctuations in pond water [23]. Elovaara (2001) [27] determined that values above 100 mg/L $CaCO_3$ as a suitable index for *L. vannamei* farming. The alkalinity values ranged between 10 and 25 mg/L in the present study. Calcium plays an important role in the process of molting in crustaceans and it is responsible for the hardening of the exoskeleton [28] and Magnesium plays a key role in the lipids, proteins, carbohydrates metabolism and serves as a cofactor in a large number of metabolic and enzymatic reactions [29]. The values

recorded during our period of study ranged from 80-100 ppm for calcium and 500-750 ppm for magnesium respectively. Water temperature is another important environmental factor for shrimp farming due to its influence on the metabolism of the crustacean [30], growth and survival [31, 32], oxygen consumption and molt cycle [31],and immune response [33, 34]. The minimum and maximum temperatures recorded in the present study varied between 28^0C and 31^0C. Maintenance of an adequate level of dissolved oxygen (D.O) in pond water is crucial for shrimp survival and prolonged exposure to lower D.O levels can inhibit shrimp growth [35, 36]. The D.O values considered suitable for development of shrimp farming fall between 4 and 6 mg/L whereas, growth delay and stimulation of mortality occurs with << 2.0 mg/L [37]. However, chronically low D.O levels can reduce the growth, feeding and molting frequency [38-40]. The D.O values ranged between 3.0 and 5.6 mg/l in the present study.

High concentration of ammonia affects shrimp growth, molt, oxygen consumption and ammonia excretion [41] [42]. Ammonia is very toxic to aquatic animals and can cause impairment of energy metabolism and damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and molluscs [43] [44]. The Ammonia values recorded in the present study ranged between 0.03 and 0.24 mg/l. Hollerman & Boyd (1980) [45] have suggested that nitrite originates from the reduction of nitrate by bacteria in anaerobic mud or water. Chen & Chin (1988) [46] estimated the safe level of nitrite in water to be 0.11mg/l for nauplii. Nitrite values recorded in the present study ranged from 0.02 to 0.48 mg/L $N\text{-}NO_2$. Similarly, the values for nitrates in the present study ranged between 0.13 and 0.38 mg/l $N\text{-}NO_3$. Esteves (1998) [47] has reported that phosphorus acts particularly in metabolic processes of living beings, such as energy storage and structure of the cell membrane. Silicates in water are essential for diatoms to carapace formation. The lowest and highest phosphates recorded were values 0.02 and 2.06 mg/l respectively. Silicate values in the present study have ranged from 10.07 to 20.12 mg/l.

The cause of swollen hindgut syndrome (SHG) could be due to bacterial infections and poor water quality [14, 48, 49]. The present study revealed that, Temperature and pH were found to have been influencing the SHG occurrence in shrimp hatcheries. The bacterial isolates belonging to *V.harveyi, V.alginolyticus and V.campbellii* were consistently isolated from SHG affected samples indicating their role in causing SHG in shrimp post larvae [14]. *Vibrio alginolyticus* and *Vibrio harveyi* were isolated from the SHG affected individuals by Aftabuddin and Akter (2011) [48] and *V. harveyi, V. alginolyticus and V. parahaemolyticus* by Sakkaravarthi *et al*., (2010) [50]. In the present study, *V. harveyi* and *V. alginolyticus* were isolated from the SHG affected individuals. SHG isolates were found to be highly sensitive to Chloramphenicol and Gentamycin [14]. Aftabuddin and Akter (2011) [48] found out that all the SHG isolates were sensitive to Oxytetracycline (OTC), Norfloxacin and Ciprofloxacin. The two isolates obtained in the present study were highly sensitive to Ciprofloxacin and Gentamycin. Histopathological studies revealed pronounced changes in Hepatopancreas and gut. Degeneration and vacuolization of the HP tubules, swollen caecae at the dorsal end of the mid gut besides the excessive growth of the muscle layers, leading to reduced lumen. The outbreak of SHG could be due to the differences in the water quality parameters and the associated bacteria.

5. Conclusion

Survey revealed that 12.50 % and 17.85 % of the shrimp hatcheries have been facing the problem of Swollen Hind Gut (SHG) in Kakinada and Visakhapatnam coasts respectively. The present study revealed that, Temperature and pH were found to have been influencing the SHG occurrence in shrimp hatcheries. *V. harveyi* and *V. alginolyticus* were isolated from the SHG affected individuals. Histopathological studies revealed pronounced changes in Hepatopancreas and gut. Degeneration and vacuolization of the HP tubules, swollen caecae at the dorsal end of the mid gut besides the excessive growth of the muscle layers, leading to reduced lumen. Strict quarantine measures besides best management practices need to be adopted for sustainable production by minimizing the disease outbreaks.

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