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Microsporidean Disease In Shrimp Culture Ponds Due To Water Parameters

Dr. Ch. Krishnamurty[1*], Dr. Ch.V.Satyanarayana Murthy[2], Tulasi Lalam[3]
Prof. B.V. Sandeep[4]
[1*]*Department of Zoology, Andhra University, Visakhapatnam*
[2]*Lecturer in Zoology, Govt. Junior College, Ponduru, Srikakulam*
[3]*JL in Zoology, Govt. Jr College Rolugunta Anakapalli Dt.*
[4]*Department of Zoology, Andhra University, Visakhapatnam*

***Corresponding Author:** Dr. Ch. Krishnamurty*
*cheepurupallimurthy@gmail.com

Abstract

The microsporidian species are typically defined by the size and shape of the spores released by vertebrate hosts. These are previously thought to be multicellular protozoan's are to be considered animals by many scientists. Recent molecular studies suggest that they are related to fungi. The examination of culturable fresh water prawn *Litopenaeus vannamei* east and west Godavari districts, Andhra Pradesh India. Reviled the presence of the on microsporidian *Agmasoma sp* in *Litopenaeus vannamei* which were collected from February-2018 to February-2020. Spores were spherical to ovoid and measured 1-1.5µm in size. They were present on the Visceral muscles; the infected Visceral muscle was damaged only at the site of infection. The spores appeared only as an outgrowth on the muscles. It had been seasonal effects on culture ponds due to may cause microsporidian diseases to analysed by the statistical and scientific methods can be calculate with the help of SPSS software. Correlation and Multiple regressions were analysis between water parameters and microsporian disease in shrimp culture ponds.

Keywords: Protozoa, Microspora, Fungi, Microsporidian, Litopenaeus vennamei, Agmasoma sp. Correlation and Multiple regression.

INTRODUCTION

The microsporidia has experienced numerous changes since the first simple system was proposed by Thelohan in 1982. This has been necessary because each new system or each revision proved to be i was allowed to be a more or less short period of time. The crunches have been to two general classes, those essential in the employment of unsound principles and those that automatically came into being when new kinds of microsporidia were discovered or old taxa characterized.

When modifications in the classification were proposed, in an effort to accommodate the new information, these changes sometime involved genuine improvements. To often, however, they compounded the difficulties either because they were merely patchwork alterations of a basically unsound system or because they actually involved abandonment of sound features or introduction of unsound ones. Rarely has there been a revision that was thorough and that, at the same time, constituted a general improvement of previous ones.

The problems of classification become increasingly acute. During the last quarter or a century, due largely to an upsurge of interest in the microsporidia and the employment of modern techniques of research, new and

different forms have been discovered at an accelerated rate while several of the previously known forms were found to possess characters that could not be accommodated in the classification then in use.

METERIAL AND METHODS

During the study, Penaeid shrimps were collected at regular monthly intervals for a period of two years during 2018-2020 at two selected locations. Host specimens were collected randomly sample from the culture ponds Kakinada and Eluru, Godavari districts Andhra Pradesh and had taken alive to the lab or in moribund condition. In the present investigation, penaeid shrimp of species and among known important penaeid shrimp namely, *Litopenaeus vannamei* (White prawn).

As soon as the samples(Shrimps) were examined with the hand lens to observe if any external infection was noticed. And observed under the a binocular microscope in the laboratory. When infection was not detected externally, the shrimp were dissected out and the different internal organs such as muscles, Hepatopancreas and intestine were examined to detect infection. When infections were fond present it has evident in the spores. When infection was present, the spores were isolated from the host tissue and smears were prepared from them, fixed; with acetone-free methyl alcohol and stained with Giemsa after an initial 1N HCl hydrolysed for 10 minutes at 60^0 C. Used stains for the Smears wet-fixed with alcoholic Bouin's fluid or Schaudinn's, Carnoy's and Heidenhain's iron haematoxylin by the stained or treated according to Feulgen's techniques. Some spores along with the tissue were fixed along with the host tissue in aqueous or alcoholic Bouin's fluid and paraffin sections were cut at 8 µm thickness. They were stained with Heidenhains iron haemotoxylin or Azan technique for histological studies. For the polar filament a drop of Hydrogen peroxide (H_20_2) had added to the air-dried smears and 90% of the spores were extruded the polar tube, it was clearly observed under dark ground illumination as well as by negative staining with India ink.

Agmasoma sp. parasitic in the muscle and hepatopancreas of Shrimps was chosen for a detailed study. Histochemical observations were carried out by fixing bits of infected tissues showing spores in different fixing fluids such as aqueous Bouin's fluid, alcoholic Bouin's fluid, Carnoy's fluid, 10% formalin, cold absolute alcohol or formal calcium, sectioned at 8µm thickness and staining them with appropriate stains. For the cytochemical observations on the spores, smears were prepared in the usual manner, fixed in various fixatives as mentioned above and stained suitably. All the methods were followed from Pearse (1968) unless otherwise mentioned.

The histochemical techniques employed are Periodic - Acid Schiff (PAS) method for the detection of carbohydrates and various other materials, and followed by PAS after acetylation and deacetylation for control of 1:2 glycol groups. PAS after diastase digestion and Best's carmine were used for detection of glycogen. For the detection of mucopolysaccharides, Steedman's Alcian blue and dialysed Iron methods were used. Metachrornatic methods such as Toluidine blue and alcoholic Toluidine blue were also used.

For the detection of various types of proteins the following tests were employed. Mercuric Bromophenol blue for the detection of basic proteins, Millon's reaction (Baker, 1956) for proteins containing tyrosine, Azur-Schiff (Kasten, 1957), Ninhydrin/Schiff (Yasuma and Itchikawa, 1953) and Chioramine T/Schiff for the demonstration of general proteins.

WATER QUALITY MANAGEMENT

The water quality parameters were very important in shrimp culture ponds. In this, an attempt has been made to focus some of the key factors where farmers have to bestow their personal attention. In fact, water quality in shrimp culture refers to the quality of water that enables successful survival and growth of shrimp. Concomitant to these, the ultimate yield is influenced by several environmental parameters and culture practices. High stocking density of shrimp in ponds which is a common practice in *vannamei* culture will eventually result in problems like deterioration of water quality as well as pond bottom. Each aqua farmer should properly manage the water quality parameters in order to attain higher production and should see that these parameters will not have an adverse impact on the surrounding environment.

The following are some important water quality parameters that the farmers should always monitor and attend to them meticulously:

The water samples have been collected from two different stations of the culture ponds from Kakinada, East Godavari and Eluru, West Godavari. Water sample has been collected from column region of the ponds in 500ml polypropylene bottles. Studies of water quality parameter have been started from Jan -2018. Water samples were collected for the determination of salinity, dissolved oxygen and pH following standard analytical procedures (APHA, 1971). Temperature and pH were measured on field.

Temperature, salinity, pH, dissolved oxygen, alkalinity, sunshine
The data has analyzed through computer by using S.P.S.S. (Statistical Package for social science) 20th version package. In analyzing the data apart from tabular analysis with Mean, Median, Std. Dev., Std. Err., Range, Variance, Minimum, Maximum and percentages, different statistical techniques are used at appropriate places. Correlation used to find out the association and direction between the parasites and parameters and multiple regression analysis will be used particularly to find out the prevalence of Physico-Chemical parameters on parasite of *Agmasoma sp.*

II. Agmasoma Sp. (=Thelohenia)
Heavily infected shrimps appeared milky white and opaque. Such shrimps when maintained in the laboratory died in a week. Smears were prepared to study the morphology and life history of the parasite. Pansporoblasts in the fresh condition measured 10.3-12.8µm (n=20) which produced 8 spores. Spores in fresh condition measured 2.7-3.0 x 4.7 – 5.2 µm (average 2.8 x 4.9µm, n=50). The polar filament which was released by the addition of a drop of Hydrogen peroxide (H_2O_2) measured 100 to 160µm in length and was uniformly thin. The parasite is assigned to the genus *Agmasoma sp. (=Thelohania)* based on its morphological characters.

Spores were spherical to ovoid and measured 1-1.5µm in size. They were present on the Visceral muscles; the infected Visceral muscle was damaged only at the site of infection. The spores appeared only as an outgrowth on the muscles.

There was diversity in morphology size of spore occurring in the shrimps. The spore structure and the appearance of spores in the body muscle tissue have shown very close affinity to the genus *Agmasoma sp*. Schizonts spherical and measured about 5.9 µm and stained with Giemsa showed 4-8 nuclei. The nuclei were deep purple against of blue cytoplasm.

Saprogenic stages showed 8 sporoblast where in nuclei are lightly stained. Spores occurring in pansporoblast. They are oval or ellipsoidal in shape. The mature spores are elongated. The spore wall was thick with a deeply stained sproplasm having a single deeply stained nucleus. A metachromatic granule was seen at posterior end of the spore. The spore measured 2.2-3.4 µm in length and 0.7- 0.8 µm width .The polar tube showed 5-8 coiles with in the spores and when extruded it measured about 105-125 µm.

The infected shrimps have become weakened or paralyzed. They also showed behavioural changes. The food intake of the diseased shrimps was reduced to great extrant. The in-activeness in the shrimps in its general behaviour was one of the major symptoms of the disease. The infection resulted in massive destruction in the muscle tissue this resulted in some destruction and replacement of tissue by the parasite. Due to excessive proliferative activity of the parasite, it resulted in the production of numerous spores. Due to this massive invasive activity lyses and necrosis of the muscle tissue was noticed. This resulted in white opaque multiple spores like structures. Due to which the shrimp disease is known as cotton shrimp disease or milky shrimp disease.

The pathogenic protozoan infects and replaces striated muscle, causing it to become opaque and white. The muscle of such shrimp appears cooked. In severely affected shrimps, the exoskeleton appears bluish black, and white tumour-like swelling may be found on the gills and sub cuticle. A few species infect gonads, haemolymph vessel, hepatopancreas and produce enlarged gonads.

Based on gross signs and symptoms, the disease can be tentatively diagnosed. Microscopic examination of squash preparation or impression smears stained with Giemsa will reveal large number of microsporidian spores. Spore characteristics may vary from species to species.

Affected animals should be destroyed and buried away from the farm. Before stocking, the possible conditioning host/intermediate host should be eliminated.

Agmasoma sps

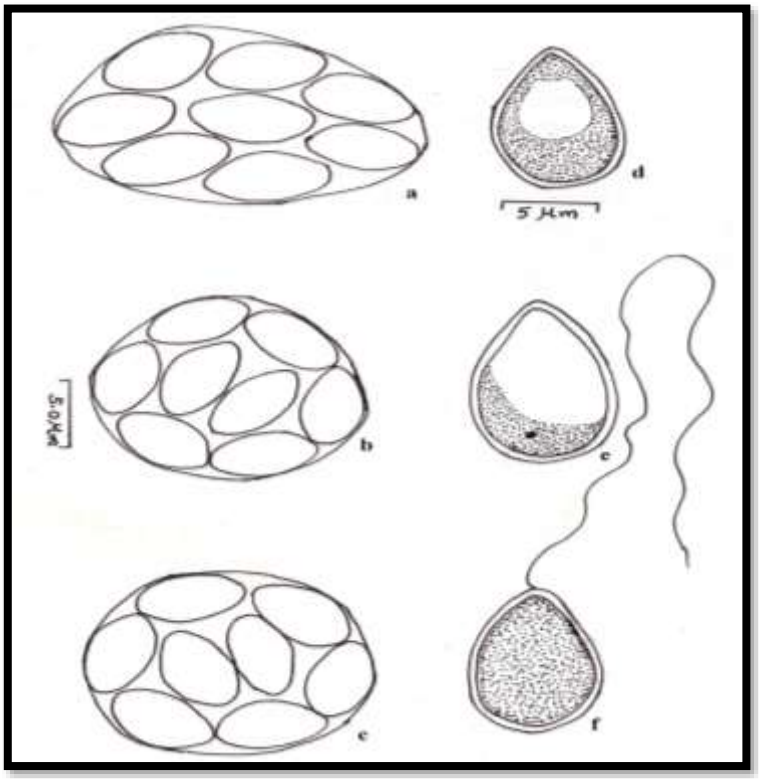

Fig. a,b and c- Pansporoblasts Fig. d. Fresh spore. Fig. e. Spore treated according to the Feulgen's technique. Fig. f. Spore showing an extruded polar filament.

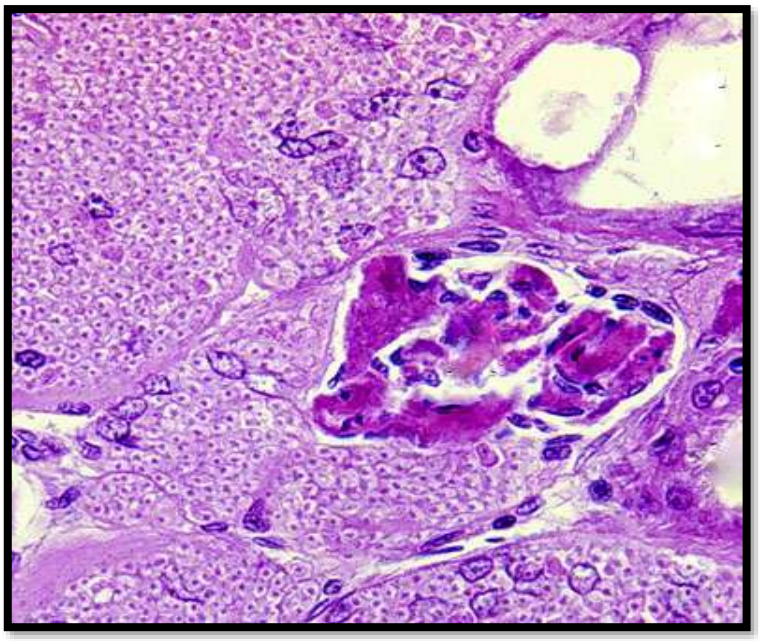

Plate: 1 Parallel histological sections of a post larvae *L. vannamei* infected with *Agmasoma sp.* (= *Thelohania sp.*) and stained with three different stains. The parasite has infected the connective tissues of the muscle and connective tissues of the hepatopancreas. The parasite infection has resulted in a marked hypertrophy of all infected cells, resulting in a compression atrophy and necrosis of the otherwise un-infected hepatopancreas tubules.

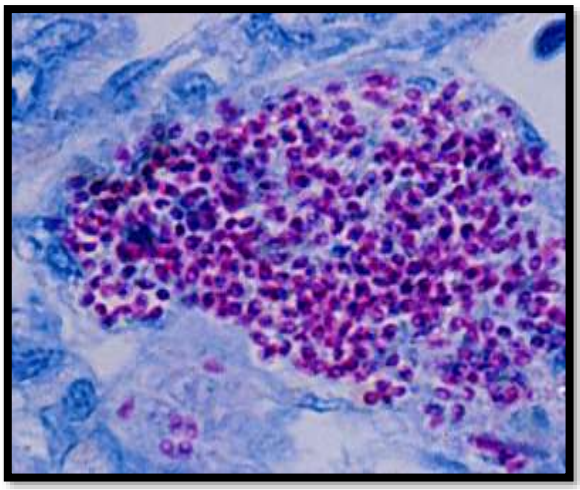

Plate: 2 The vegetative and pre-spore stages of the microsporidian are well shown with H&E, but the spore stage is chromophobic (stains poorly with H&E). In contrast, Giemsa (spores stain blue) and acid-fast stains (spores stain bright red) demonstrate very well the spore stage of the parasite.

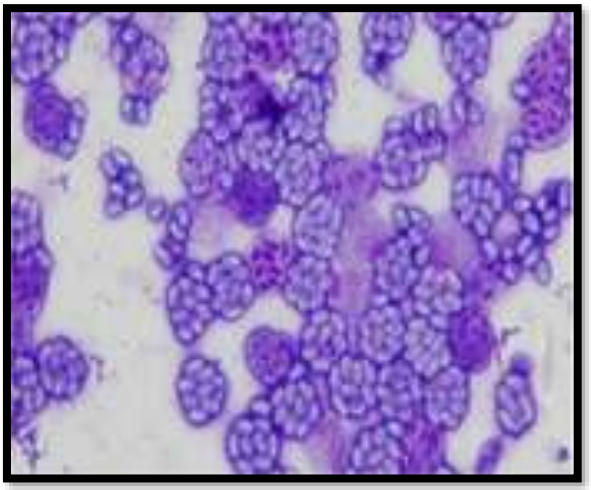

Plate: 3 Stained tissue smear showing group of 8 spores of *Agmasoma sp.* (=*Thelohania sp.*)

Table-1: Percentage distribution of microsporidian parasites in relation to hydrographical conditions of Station-1 (Culture ponds from Kakinada district) pooled data of during the study period of 2018-2020.

Month	Water Temperature (ºC)	Sunshine (hrs)	alkalinity	Salinity (‰)	Dissolved oxygen (mg/l)	pH	Percent infection of *Agmasoma sp.*
FEB	28.15	8.45	339.5	4.5	3.3	8.3	3
MAR	33.15	7.9	372	4.55	4.35	8.15	2.85
APR	34.25	8.5	362.5	5.2	4.65	8.2	4.35
MAY	35.75	7.65	384	6.5	4.65	8.2	9
JUNE	36	6.25	359	7.2	4.75	7.8	14.7
JULY	34.5	3.4	476.5	6.15	7	8	21.35
AUG	32.5	4.25	435	5.35	7.25	7.5	38
SEP	31.75	5.75	397.5	5	11.5	6.55	36.35
OCT	31.75	6.6	400	4.65	12	7.1	39.65
NOV	30.25	7.9	345	4.1	11	6.85	31.35
DEC	30	7.05	380	4.25	8.35	7.3	3
JAN	28.85	7.95	380	3.1	3.75	7.55	1.5
Descriptive statistics							
N	12	12	12	12	12	12	12
Mean	32.24	6.8	385.92	5.05	6.88	7.63	17.09
Median	32.13	7.35	380	4.83	5.88	7.68	11.85
Std. Dev.	2.6	1.64	38.51	1.13	3.17	0.58	15.42
Std. Err.	0.75	0.47	11.12	0.33	0.91	0.17	4.45
Range	7.85	5.1	137	4.1	8.7	1.75	38.15
Variance	6.74	2.7	14.27	1.28	10.03	0.34	237.72
Minimum	28.15	3.4	339.5	3.1	3.3	6.55	1.5
Maximum	36	8.5	476.5	7.2	12	8.3	39.65

Relation between Physico–Chemical parameters and seasonal incidence Microsporidian Parasites (Fungi (Protozoa): microspora) of Litopenaeus vannamei shrimp Station (Culture ponds from Kakinada).

During year 2018 to 2019 the monthly temperature ranged from a minimum of 28.15^{0}C (feb-2018) to a maximum of 36^{0}C (June-2018) (Table-1) and during year 2019 to 2020, the monthly mean temperature ranged from a minimum of 25.8^{0}C (Feb 2019) to a Maximum of 37^{0}C (June-2019) (Table-1). Seasonally, water temperature was relatively high during March, April, May and June corresponding to summer months and low during December, January and February the winter months. Alkalinity was in general high and ranged from 320 ppm (April 2018) to 420 ppm (October 2018) and the mean relative alkalinity 363ppm was recorded during 2018 to 2019 (Table-1) and during 2019 to 2020 the monthly mean relative alkalinity ranged from a minimum of 320 ppm (November 2019) to maximum of 568 ppm (July 2019) mean relative alkalinity 408 ppm (Table-1). During the study period mean salinity per cent from the pooled data ranged from 3.10 to 7.20 percent, pH ranged from 6.55 to 8.30 and dissolved oxygen ranged from 3.30 to 12.00 (mg/lit). In general, at Culture ponds from Kakinada are heaving clouded to overcast during south-west monsoon period from pooled data varied form minimum of 3.40 hrs (July) to a Maximum of 8.50 (April) and the Mean relative sun shine 6.80 hrs.

Relationship between Physico-Chemical parameters and disease prevalence of *Agmasoma sp.* of *Litopenaeus vannamei* shrimp during year 2018 -2020 at Station-1 (Culture ponds from Kakinada).

The disease prevalence of *Agmasoma sp.* on penaeid shrimp (*vannamei*) in two consecutive years for the period from February 2018 to January 2019 and February 2019 to January 2020 was recorded at Station–1 (Shrimp culture ponds form Kakinada) and presented in (Table-1). The results of Year 2018 -2019 observation showed that maximum incidence of *Agmasoma sp.* 41.30 per cent. infestation of was recorded on penaeid shrimp (*vannamei*) during October and minimum incidence was recorded 0.00 percent during February.(Table-1).

Agmasoma sp. Infestation was first recorded in February during year 20118. Per cent disease infestation showed and increasing trend (3.00 per cent) from March and continued up to November, reaching the peak during October (41.30 percent) (Table-1). Which declined thereafter and a minimum population of zero per cent was observed on February-2019 (Table-1).

Relationship between Physico-Chemical parameters and disease prevalence of *Agmasoma sp.* of *Litopenaeus vannamei* shrimp during year 2018 - 2019 and 2012 – 2020 at Station – 2 (Culture ponds from Eluru).

The disease prevalence of *Agmasoma sp.* on penaeid shrimp in two consecutive years for the period from February 2018 to January 2019 and February 2019 to January 2020 was recorded at Station – 2 (Shrimp culture ponds form Eluru) and presented. The results of Year 2018 -2019 observation showed that maximum incidence of *Agmasoma sp.* 42.00 percent infestation of was recorded on penaeid shrimp (*vannamei*) during October and minimum incidence was recorded 0.00 percent during February 2018. (Table-1).

Agmasoma sp. Infestation was first recorded in February during year 2018. Percent disease infestation showed and increasing trend (5.70 per cent) from March and continued upto November, reaching the peak during October (42.00 percent). Which declined thereafter and a minimum population of zero per cent was observed on February-2018 (Table-1).

Table-3: Correlation matrix of the relationship between Physico- Chemical parameters and disease prevalence *Agmasoma sp.* on *Litopenaeus vannamei* shrimps at St.II (Culture ponds, Eluru, West Godavari district) during 2018-2019.

	Y	X_1	X_2	X_3	X_4	X_5	X_6
Y Agmasoma sp.(%)	1						
X_1 Water Temperature (ºC)	-.273	1					
X_2 Sunshine (hrs)	**-.629***	.296	1				
X_3 Alkalinity	.345	-.097	-.267	1			
X_4 Salinity (‰)	-.088	.772**	-.039	.227	1		
X_5 Dissolved oxygen (mg/l)	**.727****	-.415	-.252	.620*	-.297	1	
X_6 pH	-.562	.867**	.372	-.230	.749**	-.709**	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

In the correlation analysis *Agmasoma sp.* is considered as dependent variable (Y) and Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6) are chosen as independent variables. Water Temperature (X_1), Sunshine (X_2), Salinity (X_4) and pH (X_6) variables are associated negatively with the *Agmasoma sp.* variable. In this Sunshine (X_2) variable is statistically significant at 5 per cent level. Alkalinity (X_3), and Dissolved oxygen (X_5) variables are positively correlated with *Agmasoma sp.* variable. One independent variable - Dissolved oxygen (X_5) is statistically significant at one per cent level.

Table-4: Multiple regression analysis of the disease prevalence *Agmasoma sp.* (Y) on *Litopenaeus vannamei* shrimps with Physico-chemical parameters (X) and at St. II (Culture ponds, Eluru, West Godavari district) during 2018-2019

Parameters	B	Std. Error	t-value	Sig.
(Constant)	52.859	69.576	.760	.482
X_1 Water Temperature (ºC)	.916	2.947	.311	.768
X_2 Sunshine (hrs)	-3.396	1.767	-1.922	.113
X_3 Alkalinity	-.139	.100	-1.394	.222
X_4 Salinity (‰)	3.435	5.019	.684	.524
X_5 Dissolved oxygen (mg/l)	3.481	1.811	1.922	.113
X_6 pH	-4.201	13.754	-.305	.772
Model Summary				
R Square	**.836**			
Adjusted R Square	.639			
F-value	4.240			

Dependent Variable: Agmasoma sp.(%).

Y= 52.859 + .916X_1 + -3.396X_2 + -.139X_3 + 3.435X_4 + 3.481X_5 +-4.201X_6

Multiple linear regression technique is used to analyse the impact on *Agmasoma sp.* by the explanatory parameters-Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6). Water Temperature (X_1), Salinity (X_4) and Dissolved oxygen (X_5) parameters have positive effect on *Agmasoma sp.* and the remaining parameters have negative effect. None of the independent variables is statistically significant (Table-4).

The model fitness is represented by R-square value. The R-square value shows that the percentage of change in *Agmasoma sp.* explained by the explanatory parameters together through this model. In this model the R-square value explained 83.6 percentage of changes in *Agmasoma sp.* together by all the explanatory parameters (Table-4).

The model concluded that Water Temperature (X_1), Salinity (X_4) and Dissolved oxygen (X_5) have positive incidence on *Agmasoma sp.* among the selected independent parameters. The model further reveals that 83.6 percentage of prevalence is notified on *Agmasoma sp.* with together all parmeters (Table-4).

Table-5 Correlation matrix of the relationship between Physico- Chemical parameters and disease prevalence *Agmasoma sp.* on *Litopenaeus vannamei* shrimps at St.II (Culture ponds, Eluru, West Godavari district) during 2019-2020.

	Y	X_1	X_2	X_3	X_4	X_5	X_6
Y Agmasoma sp. (%)	1						
X_1 Water Temperature (ºC)	.031	1					
X_2 Sunshine (hrs)	-.494	-.413	1				
X_3 Alkalinity	**.674***	.192	-.242	1			
X_4 Salinity (‰)	.501	.566	-.901**	.185	1		
X_5 Dissolved oxygen (mg/l)	-.165	-.079	-.154	-.263	.063	1	
X_6 pH	**-.654***	.068	.296	-.802**	-.241	.247	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

In the correlation analysis *Agmasoma sp.* is considered as dependent variable (Y) and Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6) are chosen as independent variables. Sunshine (X_2), Dissolved oxygen (X_5) and pH (X_6) variables are associated negatively with the

Agmasoma sp. variable. In this pH (X_6) variable is statistically significant at 5 per cent level while Water Temperature (X_1), Alkalinity (X_3), and Salinity (X_4) are positively correlated with *Agmasoma sp.* variable. Here one independent variable - Alkalinity (X_3) - is statistically significant at 5 per cent level (Table-5).

Table-6: Multiple regression analysis of the disease prevalence *Agmasoma sp.* (Y) on *Litopenaeus vannamei* shrimps with Physico-chemical parameters (X) and at St. II (Culture ponds, Eluru, West Godavari district) during 2019-2020.

Parameters	β	Std. Error	t-value	Sig.
(Constant)	-154.696	250.491	-.618	.564
X_1 Water Temperature (ºC)	-4.162	2.280	-1.825	.128
X_2 Sunshine (hrs)	2.222	4.141	.537	.615
X_3 Alkalinity	.331	.167	1.984	.104
X_4 Salinity (‰)	6.989	4.198	1.665	.157
X_5 Dissolved oxygen (mg/l)	-.437	1.533	-.285	.787
X_6 pH	16.441	29.380	.560	.600
Model Summary				
R Square	**.771**			
Adjusted R Square	.497			
F-value	2.809			

Dependent Variable: Agmasoma sp.(%).

Y= -154.696 + -4.162X_1 + 2.222X_2 + .331X_3 + 6.989X_4 + -.437X_5 + 16.441X_6

Multiple linear regression technique is used to analyse the impact on *Agmasoma sp.* by the explanatory parameters-Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6). Sunshine (X_2), Alkalinity (X_3), Salinity (X_4) and pH (X_6) parameters have positive effect on *Agmasoma sp.* and the remaining parameters have negative effect. None of the independent variable coefficient is statistically significant (Table-6).

The model fitness is represented by R-square value. The R-square value shows that the percentage of change in *Agmasoma sp.* explained by the explanatory parameters together through this model. In this model the R-square value explained 77.1 percentage of changes in *Agmasoma sp.* together by all the explanatory parameters (Table-6).

The model concluded that Sunshine (X_2), Alkalinity (X_3), Salinity (X_4) and pH (X_6) have positive incidence on *Agmasoma sp.* among the selected independent parameters. The model further reveals that 77.1 percentage of prevalence is notified on *Agmasoma sp.* with together all parameters (Table-6).

Table-7: Correlation matrix of the relationship between Physico- Chemical parameters and disease prevalence *Agmasoma sp.* on *Litopenaeus vannamei* shrimps at St.II (Culture ponds, Eluru, West Godavari district) Pooled data of year 2018-2019 and 2019-2020.

	Y	X_1	X_2	X_3	X_4	X_5	X_6
Y ***Agmasoma sp.*** (%)	1						
X_1 Water Temperature (ºC)	-.138	1					
X_2 Sunshine (hrs)	**-.670***	.164	1				
X_3 Alkalinity	**.626***	.085	-.317	1			
X_4 Salinity (‰)	.293	.618*	-.462	.299	1		
X_5 Dissolved oxygen (mg/l)	**.828****	-.503	-.480	.479	-.096	1	
X_6 pH	**-.627***	.754**	.403	-.432	.332	-.833**	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

In the correlation analysis *Agmasoma sp.* is considered as dependent variable (Y) and Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6) are chosen as independent variables. Water Temperature (X_1), Sunshine (X_2) and pH (X_6) variables are associated negatively with the *Agmasoma sp.* variable. Among these, Sunshine (X_2) and pH (X_6) variables are statistically significant at 5 per cent level. Alkalinity (X_3), Salinity (X_4) and Dissolved oxygen (X_5) are positively correlated with *Agmasoma sp.* variable. Two independent variables viz., Alkalinity (X_3) and Dissolved oxygen (X_5) are statistically significant at five and one per cent levels (Table-7).

Table-8: Multiple regression analysis of the disease prevalence *Agmasoma sp.* (Y) on *Litopenaeus vannamei* shrimps with Physico-chemical parameters (X) and at St. II (Culture ponds, Eluru, West Godavari district) Pooled data of year 2018-2019 and 2019-2020.

Parameters	β	Std. Error	t-value	Sig.
(Constant)	-47.948	106.151	-.452	.670
X_1 Water Temperature (ºC)	2.551	2.335	1.092	.324
X_2 Sunshine (hrs)	-1.925	1.747	-1.102	.320
X_3 Alkalinity	.028	.100	.278	.792
X_4 Salinity (‰)	.535	2.643	.202	.848
X_5 Dissolved oxygen (mg/l)	7.247*	3.258	2.225	.077
X_6 pH	-7.920	14.823	-.534	.616
Model Summary				
R Square	**0.888**			
Adjusted R Square	0.753			
F-value	6.596			

Dependent Variable: Agmasoma sp.(%). *. Significant at the 5% level.

Y= -47.948 + 2.551X_1 + -1.925X_2 + .028X_3 + .535X_4 + 7.247*X_5 + -7.920X_6

Multiple linear regression technique is used to analyse the impact on *Agmasoma sp.* by the explanatory parameters Water Temperature (X_1), Sunshine (X_2), Alkalinity (X_3), Salinity (X_4), Dissolved oxygen (X_5) and pH (X_6). Water Temperature (X_1), Alkalinity (X_3), Salinity (X_4) and Dissolved oxygen (X_5) parameters have positive effect on *Agmasoma sp.* and the remaining parameters have negative effect. Due to one per cent change in Dissolved oxygen (X_5), *Agmasoma sp.* increased by 7.247 percentage. It is statistically significant at 5 per cent level. (Table-8).

The model fitness is represented by R-square value. The R-square value shows that the percentage of change in *Agmasoma sp.* explained by the explanatory parameters together through this model. In this model the R-square value explained 88.8 percentage of changes in *Agmasoma sp.* together by all the explanatory parameters (Table-8).

The model concluded that Water Temperature (X_1), Alkalinity (X_3), Salinity (X_4) and Dissolved oxygen (X_5) have positive incidence on *Agmasoma sp.* among the selected independent parameters. The model further reveals that 88.8 percentage of prevalence is notified on *Agmasoma sp.* with together all parameters (Table-8).

DISCUSSION:

Data obtained by monthly sampling revealed some differences in the seasonal prevalence of the microsporidian parasite in the target Litopenaeus vannamei shrimp and the prevalence and intensity of microsporidian parasite of these three species were outstanding among two selected study locations. The observations of these three species of microsporidians were recorded in the Vannamei shrimp muscle during study period was observed that highest prevalence of microsporidians occurred during end of the dry season (June to November) and the levels of infections decreased during December to May, which characterized by temperature, salinity and dissolved oxygen.

Gunalan Balakrishnan et al., (2011), the reported on the culture of L. vannamei in the brackish water shrimp farm in Bhimavaram, west Godavari district, Andhra Pradesh, India. This study shows that stocking density affects growth of L. vannamei. Several authors have reported on the growth and survival of L. vannamei in different salinities and densities. The pH of pond water is influenced by many factors, including pH of source water, acidity of bottom soil and shrimp culture inputs and biological activity. The recommended favourable pH range is 7.6-8.6 for L. vannamei. The concentrations of Dissolved Oxygen (DO) in all ponds ranged from 3.5- 5.5 mg/l in the morning and 4.5-10.2 mg/l in the evening during the culture period. The values of water quality parameters reveal that all these are in the acceptable range for survival and growth for L. vannamei. The growth of the shrimps depends on the quality of feed. In the present study it was noticed that CP feed, (Charoen Pokphand Feed India Pvt. Ltd.,) was used for all the ponds and the amount was followed as per feed chart Jayati Chakraboti and Probir K. Bandyapadhyay (2009), reported on "Seasonal incidence of protozoan parasites of the black tiger shrimp (Penaeus monodon) of Sundarbans, West Bengal, India". There is a delicate balance between the host, pathogen and environment.

Limsuwan et al., (2008), Limsuwan, (1991), Flegel et al., (1992) reported P. monodon the shrimp were first observed to be infected with microsporidian parasites between 20-25 days after stocking the PL into the grow out ponds. Affected shrimp had a whitish or milky appearance on various parts of the body. As the shrimp grew larger, this clinical sign was more easily observed especially dorsally from the hepatopancreas to the middle of the body.

Lightner (1993), Kautsky et al., (2000), Gualteros Rodriguez (2003), Rhode 1992; Jayasree et al., (2001), reported prevalence of the Protozoan parasites was observed in summer following the same seasonal trend as ciliates. Iversen and Kelly, (1976) reported on the prevalence of infection reached 28.5% in pond 3 after 60 days post stocking. Later during the culture period, despite infected shrimp being eaten by healthy shrimp, the prevalence of infection did not increase. However, microsproridiosis was successfully transmitted experimentally in pink shrimp, P. duorarum PL, by feeding them on the faces of spotted sea trout (Cynoscion nebulosus) that had been fed infected shrimp.

The observations made in the present study show that the percentage infection was maximum in the winter months of November to January and minimum in summer months of April to June. This is probably because of the variations observed that the temperature to be 210C and 15°C-180C probably the highest during the year in the temperate countries occur in summer months, while in tropical countries the corresponding temperatures are in the winter season (around 210C). This clearly shows that the ideal temperature for microsporidian infections ranged between 180C - 210C but there lower 'nor at higher temperatures. Probably the pattern of life cycle changes to suit these ecological conditions. The period when no apparent infection was observed the present study is during May to June which is probably the time
taken for the parasite to restart the infection and to develop into visible microsporidia spores as is the case in G. hertwigii (Delisle,1969). A strange correlation between the growth, maturity of the host and the development rate and incidence of parasite has been observed. The parasites increase in numbers simultaneously with the growth of the host, and by the time the host reaches maturity it shows fully formed (usually largest) xenomas. The winter temperature apparently being favourable for the host and conducive for the growth of the parasite.

CONCLUSION:

The present study, carried out for a period of two years during 2018-2019 and 2019-2020 at two diverse habitats along the east coast of India namely, culture ponds from Kakinada, East Godavari and Culture ponds from Eluru, West Godavari Districts, Andhra Pradesh, India was aimed at investigation the biology of the three known microspora parasites of penaeid shrimps (*Litopenaeus vannamei*) vis-à-vis environmental conditions. The rational has been that the study, which takes into account the host parasite environment relationships, would help to understand some complexities in shrimp culture particularly the large scale mortalities of Shrimps in culture ponds and other impoundments accentuated by overcrowding. Investigations on the ecology of pathogens included an account of their composition, prevalence, distribution, seasonal cycles in relation to abiotic factors: the highest prevalence of microsporidians occurred during the end of the dry season (June to November) and the levels of infections decreased during December to May, which characterized by temperature, in conjunction with Sunshine, Alkalinity, Dissolved oxygen and pH largely influenced prevalence of microsporidian infections.

The studies will be contribute significantly to predict the disease outbreak, disease surveillance, disease prevention and health management of juveniles, adults, and gravids in order to improve the shrimp's health in hatcheries/Culture ponds and narrow down the post larval mortality rate and economic loss.

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