ISSN 2250-3153

Prevalence of Epibiont Protozoan communities on *Penaeus monodon* (Fabricius) from the Hatchery off Visakhapatnam, East Coast of Andhra Pradesh, India

K. Ramesh Babu

Department of Marine Living Resources, Andhra University, Visakhapatnam-530 003

***Abstract*-** The present study aimed to establish the information on protozoan diseases in Black tiger shrimp Penaeus monodon during the year 2012. Due to high levels of ciliate infestations can affect respiration, feeding, growth, survival and also irritation behaviour of larvae (zoea, mysis and post larvae) was observed. Protozoan diseases in P. monodon was global problem in hatcheries mainly affect the eggs and larval stages. In current study we found different variations of ciliary infections were observed throughout the year (2012), we also concentrated on some physical parameters like PH, Temperature (to C), and salinity (ppt), variations of these parameters will affect the larval growth and survival rate in hatchery system.

***Index Terms*-** Penaeus monodon, Protozoans, ciliates and survival rate.

I. INTRODUCTION

Penaeus monodon serve as host for different kinds of protozoan parasites and commensals, occur both outside and inside the host body Peritrichous ciliates like *Zoothamnium* sp. and *Voritcella* sp. are ecto-commensals and microsporidia and gregarines are endo-commensals.

In farmed shrimp, heavy infestations of ectosymbionts are expected because transmission is enhanced due to high stocking densities and variations of environmental parameters (e.g., ammonium, nitrate, and dissolved oxygen levels), the consequence of pond fertilization (Kautsky et al., 2000; Gualteros-Rodríguez, 2003). The ectosymbionts peritrichous ciliates remain attached to the gills and limbs. A possible relationship between the peritrich ciliate, *Zoothamnium* sp., and mortality of host following stress was discussed previously (Overstreet 1973). Their abundant presence can interfere with the breathing and mobility of the host. The *Zoothamnium* infections are at their peak during monsoon and post-monsoon months and these infections appear to be governed directly by salinity of the habitat and indirectly by the rainfall (Jayasree et al. 2001).

Microbial diseases have been reported to be a major limiting factor in production both in wild and cultured shrimps. A large amount of data has recently been accumulated on a variety of pathogens affecting both larval and cultured shrimp (Overstreet, 1973, 1982; Lightner, 1983, 1985). Among the Protozoa, microsporidia have been most frequently recorded (Parsons & Khan, 1986) but the knowledge of other protozoan infecting shrimp is not so extensive. From the observation already made by different workers in the field of Pathology of *Penaeus monodon*. Bradburg and Trager (1967) were reported Excystation of apstone cilliates in relation to moulting of their crustacean hosts. Diseases caused by protozoa in the biology of crustacean was by Couch John (1983), Kruse (1959) gave detailed information about parasites of the commercial shrimps.

II. MATERIALS AND METHODS

For studies on protozoan diseases of larvae and post larvae, the larval samples were collected from each tank by means of 250 ml beaker and estimated by random sampling method for larval populations. Every day sampling was practiced for this study. For studies on the symptoms of various diseases, the larvae were observed under a research microscope and noted the physical appearance of the larvae and post larvae. The behaviour of the larvae and their feeding condition and their activity was also note down. . Observations were made on the seasonal prevalence of ciliate protozoan and in relation to survival and mortality. Further, the infected larvae and post larvae were experimented to control the infection by using different antibiotics and chemicals. The following antibiotics were used for this experiment

A) Chloramphenicol	(CP)	1.5 - 3.5 ppm
B) Prefuran	(PREF)	0.5 - 1.0 ppm
C) Treflan	(TFL)	0.05 - 0.5 ppm
D) Oxytetracycline	(OTC)	1.5 - .3.5 ppm
E) Furozolidone	(FZ)	1.5 - 3.5 ppm
F) Erythromycine	(EM)	1.5 – 2.5 ppm
G) Cifrofloxine	(CF)	1.5 - 2.5 ppm
H) Formaldehyde		5.0 - 15 ppm

III. RESULTS

Experiments were conducted on the prevalence of protozoan, diseases of *P. mondon* larvae (from Z_1 – PL – 20) for the year of 2012. Microbial diseases have been reported to be a major limiting factor in production both in hatchery and cultured shrimps

A number of ciliate protozoans occur as symbionts, commensal, parasites and pathogens of crustacean larvae. The exoskeleton of the shrimp larvae served as a substratum on which both sessile and mobile forms of peritrichous ciliates lived and reproduced. Most peritrichous ciliates viz., *Zoothammium Epistylis, Vorticella, Lagenophrys, Acineta* found to attach themselves on the cuticle, causing mortality of larvae and post-

larvae. They were observed to also interfere in feeding and respiratory activity of the larvae and post-larvae. This was the main reason found for the mortality of the shrimp larval and post-larval stages.

Site of occurrence and pathological signs of ciliate (Protozoan) diseases: The peritrichous cilliates viz *Zoothamnium* sp., *Vorticella* sp. *Epistylis* sp. and *Lagenophrys* sp. were recorded in *P. monodon's* larvae and juveniles. All the peritrichous cilliates were commonly noticed on external part of the body, on the cuticle and gills. Reddish to brownish gills were observed in case of chronic ptotozoan infections.

The affected larvae and post-larvae faced difficulties in locomotion and feeding activities. Large numbers of colonial ciliates were observed to attach themselves on the mouth parts, hindering the free movement of the mouthparts and disabling the larvae and juvenile forms to collect and feed the food material. Larvae and post-larvae were found to die due to starvation stress.

Table 1: Seasonal variations of Protozoan diseases in *Penaeus monodon* and survival of zoea larvae percentage in 2012.

Months	Stocking density in millions	Protozoan diseases		Percentage of survival after treatment	
		Millions	%	Millions	%
January	0.945	0.138	14.67	0.818	83.87
February	0.938	0.171	18.24	0.764	81.54
March	0.874	0.173	19.82	0.704	80.62
April	0.960	0.210	22.46	0.762	79.40
May	0.875	0.160	18.34	0.687	78.54
June	0.967	0.124	12.87	0.809	83.72
July	0.890	0.139	15.63	0.743	83.46
August	0.865	0.178	20.61	0.727	84.13
September	0.956	0.166	17.35	0.790	82.67
October	-	-	-	-	-
November	-	-	-	-	-
December	0.845	0.142	16.89	0.711	84.19

Table 2: Seasonal variations of Protozoan diseases in *Penaeus monodon* and survival of Mysis larvae percentage in 2012.

Months	Stocking density in millions	Protozoan diseases		Percentage of survival after treatment	
		Millions	%	Millions	%
January	0.818	0.143	17.49	0.708	86.65
February	0.764	0.166	21.74	0.668	87.55
March	0.704	0.096	13.70	0.597	84.87
April	0.762	0.142	18.62	0.639	83.88
May	0.687	0.084	12.37	0.560	81.53
June	0.809	0.167	20.66	0.708	87.56
July	0.743	0.114	15.38	0.634	85.36
August	0.727	0.085	11.74	0.632	86.82
September	0.790	0.114	14.46	0.638	80.76
October	-	-	-	-	-
November	-	-	-	-	-
December	0.711	0.128	18.10	0.584	82.25

Table 3: Seasonal variations of Protozoan diseases in *Penaeus monodon* and survival of Post larvae (PL1-10) percentage in 2012.

Months	Stocking density in millions	Protozoan diseases		Percentage of survival after treatment	
		Millions	%	Millions	%
January	0.708	0.116	16.49	0.630	89.11
February	0.668	0.145	21.74	0.593	88.90
March	0.597	0.102	17.13	0.551	92.46
April	0.639	0.119	18.62	0.552	86.38
May	0.560	0.069	12.37	0.479	85.64
June	0.708	0.146	20.66	0.633	89.36
July	0.634	0.097	15.38	0.554	87.47
August	0.632	0.086	13.74	0.558	88.28
September	0.638	0.130	20.46	0.559	87.55
October	-	-	-	-	-
November	-	-	-	-	-
December	0.584	0.106	18.10	0.513	87.82

Table 4: Seasonal variations of Protozoan diseases in *Penaeus monodon* and survival of Post larvae (PL11-20) percentage in 2012.

Months	Stocking density in millions	Protozoan diseases		Percentage of survival after treatment	
		Millions	%	Millions	%
January	0.630	0.096	15.30	0.574	91.21
February	0.593	0.134	22.67	0.546	92.17
March	0.551	0.100	18.26	0.501	90.78
April	0.552	0.118	21.47	0.485	88.02
May	0.479	0.072	15.12	0.429	89.61
June	0.633	0.118	18.65	0.579	91.53
July	0.554	0.108	19.63	0.478	86.24
August	0.558	0.118	21.26	0.482	86.52
September	0.559	0.118	12.35	0.477	85.45
October	-	-	-	-	-
November	-	-	-	-	-
December	0.513	0.075	14.63	0.465	90.54

Figure 1: Rounded area in the figure 1 infected with protozoan disease at egg stage

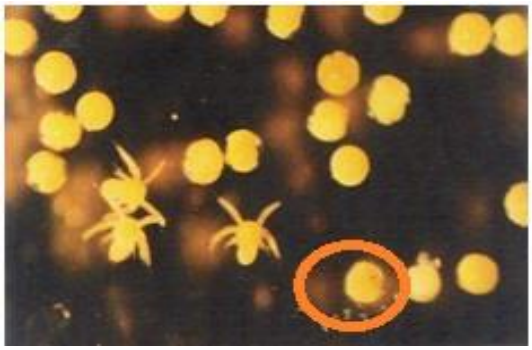

Figure 2: Marked area in figure 2 with attachment of Protozoans

Figure 3: Appendages, Uropods were infected with Protozoans at Mysis 3rd stage

Figure 4: Pleopods were infected with Protozoan diseases

Stage – Zoea: 1 – 3

Table (1) shows percentage of infected larvae and survival rate after infection due to protozoan diseases. It is evident from the table that protozoan disease have been observed throughout the year, highest percentage, 22% infected zoea was noticed in the month of April during the year 2012, lowest of 14% observed in January in the same year. The high survival rate of 83%, 83%, 83%, 83%, 82% and 84% was recorded during monsoon and post monsoon period in the year 2012 (Table 1) after treatment.

Stage - Mysis: 1 - 3

Mysis larvae were also affected with protozoan diseases, especially with the ciliate infection. The highest percentage of protozoan disease in Mysis stage was recorded as 21% and 20% in the months of February and June and lowest percentage of 11%, observed in the month of August during the year of 2012 (Table 2).

Post Larvae (PL): 1 – 10:

Incidence of infection due to protozoan ciliates, were recorded in the early juvenile stages. The contagious diseases used to spread fast in the aquatic media from larval stages to the post-larval stages. The various details about the % of juvenile shrimps infected are shown in Table 3. It was noticed in the present observations that prolonged rearing of the larvae and

continuous operation of the hatchery or laboratory without frequent time gaps between cycles lead to the development of infections. It was also observed that the juvenile stages were relatively resistant to diseases when compared to the larval forms. Various antibiotics and disinfectants were used to control the infection caused by ciliates, It was observed that the ciliates were susceptible to 1-10 ppm in various post-larval stages. Similarly 2-4 ppm of cupric sulphate was found to be useful for successful control of the ciliates in commercial operations; thorough water exchange followed each treatment.

Post Larvae (PL): 10-20:

Table 4 shows that the highest percentage 22.67% infected post larvae was noticed in the month of February and lowest of 12.35% observed in September in the same year. The high survival rate of 92.17%, 91.53%, 91.21%, 90.78%, and 89.61% was observed February, June , January, March, May respectively after treatment with antibiotics.

IV. DISCUSSION:

Disease is a major threat for any biological system against which man's fight is un ending. In an aquatic system prevention is the best remedy. If a hatchery system is infected with a disease, it is better to discontinue the operation till the pathogen is eradicated by disinfection and drying. Parasitic infections were earlier reported from different geographical areas of India (Bower et al. 1994; Prasadan and Janardan 2001; Johny et al. 2006).

Diseases and parasites of shrimp constitute a potential constraint on successful production this valuable seafood. A large amount of data has recently been accumulated on a variety of pathogens affecting both natural and cultured shrimp (Overstreet, 1973, 1982 and Lightner, 1983, 1985).

The development of protozoan infection has a direct relation to the water exchange and quality of the feeds. In good water exchange by flow through mechanism, that left over feeds and excretory materials can be removed efficiently preventing the ciliates to multiply. But in poor quality feeds which are left behind in the tank bottom and water exchange, resulting in high nutrient levels the ciliates multiply rapidly attacking the appendage of the swimming larvae. Overstreet (1973) made extensive study on *Zoothamnium* infection in penaeid shrimps and found a positive correlation between the stocking density of shrimp and the prevalence of infection.

The prevalence of the protozoan parasite was observed in summer following the same seasonal trend as ciliates. This parasite is highly pathogenic to shrimp and heavy infection may cause loss of appetite, growth retardation and ultimately death of the host in the culture system (Lightner 1993). The current studies revealed that the peritrichous ciliates infected larvae and post larvae of *P. monodon* exhibited a fuzzy appearance, frequent jumping or circular swimming movements. Similarly, earlier studies have shown that epi-commensal organisms if occurred in large numbers on the body surfaces and appendages could cause difficulties in locomotion, feeding, moulting or respiration, resulting in mortalities (Johnson et al., 1973, Chang and Su 1992). Transmission of ciliate parasites increases during summer in tropical or subtropical climates because of the physicochemical conditions of the farm (Norma et al. 2009), increasing metabolism and molting of decapods host occurred due to heavy amount of solar energy (Rhode 1992; Jayasree et al. 2001).

Infection of protozoan ciliates on the gills of the larvae usually do not effect growth because they do not derive nourishment directly from the host but considered to have a synergistic effect during the periods of stress. But the commensal ciliates are considered pathogenic because of their heavy anastamosis on the gills and mouthparts, causing the mouthparts immobile, preventing the larvae to feed actively; death was due to starvation.

From the discussion it can be concluded that, During summer months due to high intensity of solar energy the moulting process is favoured (Rhode 1992; Jayasree et al. 2001), thus incresing the invasion of parasites. So adequate measures should be taken in the particular months to avoid protozoan infections.

ACKNOWLEDGEMENT

The author thankful to "Pavan aqua Pvt. Ltd" located at Visakhapatnam for carried out this work.

REFERENCES

[1] L, Jayasree, P. Janakiram, R. Madhavi, 2001," Epibionts and parasites of *Macrobrachium rosenbergii* and *Metapenaeus dobsoni* from Gosthani estuary". J Nat Hist: 35:157–167. doi: 10.1080/00222930150215297.

[2] W. Gualteros-Rodríguez, 2003, Estudio de la comunidad de nemátodos meiobentónicos en 2 estanques de producción de camarón. Golfo de Guayaquil, Ecuador. MS thesis. Escuela Superior Politécnica del Litoral, Facultad de Ingeniería Marítima y Ciencias del Mar. Guayaquil, Ecuador.

[3] N. Kautsky, P. Rönnbäck, M. Tedengren, M. Troell, 2000 "Ecosystem perspectives on management of diseases in shrimp pond farming". Aquaculture. 191:145–161. doi: 10.1016/S0044-8486(00)00424-5.

[4] S.M. Bower, S.E. McGladdery, I.M. Price, 1994, "Synopsis of infectious diseases and parasites of commercially exploited shellfish". Annu Rev Fish Dis 4:1–199. doi: 10.1016/0959-8030(94)90028-0.

[5] P.K. Prasadan, P.K. Janardan. 2001, "Three new species of gregarines (Apicoplexa: Sporozoea: Porosporidae) in the Estuarine crabs from Kerala, India". Acta Protozool: 40:303–309.

[6] S. Johny, S. Kanginakudru, M.C. Muralirangan, J. Nagaraja, 2006, "Morphological and molecular characterization of a new microsporidian (Protozoa: Microsporidia) isolated from *Spodoptera litura* (Fabricus) (Lepidoptera: Noctuidae)". Parasitology 92(2):59–65.

[7] D.V. Lightner, Diseases of cultured peneaid shrimp. In: McVey JP, Lightner DV, editors. CRC handbook of mariculture: crustacean aquaculture. Boca Raton, FL: CRC Press; 1993. pp. 303–346.

[8] A. Norma, López-Téllez, V.M. Vidal-Martínez, R.M. Overstreet, 2009, "Seasonal variation of ectosymbiotic ciliates on farmed and wild shrimps from coastal Yucatan, Mexico". Aquaculture: 287: 271–277. doi: 10.1016/j.aquaculture.2008.11.003.

[9] K. Rhode, "Latitudinal gradients in species diversity: the search for the primary cause", 1992,. Oikos: 65:514–527. doi: 10.2307/3545569.

[10] R.M. Overstreet, 1973, "Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture. 2: 105-140.

[11] R.M. Overstreet, 1982, "Some parasitological aspects of shrimp culture in the united states. In: Parasitology and pathology of marine organisms of the World Ocean (Ed by W.J. Hargis)". 117-122 NOAA Technical Report. NM FS25.

[12] D.V. Lightner, 1983, "Disease of cultured penaeid shrimp in J.P. Mc Vey (Ed.)". CRC Hand book of mariculture vol-I. Crustacean Aquaculture, CRC Press, Bocon Raton. p. 289-320.

[13] Parson and R.A. Khan, 1986, "Microsporidiosis in the northern shrimp *Pandalus borealis*", J. Invert. Pathol. 47 :74-81.

[14] D.V. Lightner, 1985, A review of the disease of cultured penaeid shrimp and prawns with emphasis on recent discoveries and developments. Proceedings of the 1st international conference on the culture of penaeid prawns/shrimps, Iloilo city phillipoins. 1984-85. SEAFDEC Acquaculture. Dept. pp. 79-103.

[15] P.C. Bradbury and W. Trager, 1967 "Excystation of apostone ciliates in relation to moulting of their crustacean hosts. II effect of glycogen". Biol. Bull. (Woods Hole. Mass).

[16] A. Couch John, 1983, Diseases caused by protozoea in the biology of crustacean, edited by Proven zano A.J., editor in chief Blise. D.E. Vol. 6, pp. 79-111.

[17] D.N. Kruse, 1959, "Parasites of the commercial shrimps. *Penaeus aztecus* Ies. *P. duorarum.* Burken-road and *P. satiferus* (Linnaeus)". Tulane Stud. Zool. 7 :123-44.

[18] S.K. Johnson, J.C. Parker and W.H. Holcomb, 1973, Control of Zoothamnium on Penaeid shrimp. Prooceedings of the 4th Annual workshop. World Mariculture society. pp. 321-331.

[19] C.F. Chang and M.S. Su, 1992, In diseases of cultured penaeid shrimp in Asia and the United States edited by Fulk, W. & Main K.L. The Oceanic Institute Hawaii. 113.

AUTHORS

First Author – Dr. K. Ramesh Babu, Assistant Professor, Department of Marine Living Resources, Andhra University, Visakhapatnam-530 003.

Correspondence Author – email Id: krameshmlr@gmail.com