JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Presence of Protozoans parasites in Larval, and Post Larval stages of *Penaeus monodon* in a Shrimp Hatchery, Tupilipalem, Gudur, Tirupathi, Dt.

[1]B LAKSHMI PRASANNA LATHA, Department of Fishery Science and Aquaculture, SV University, Tirupathi, Andhra Pradesh, India.

[2]Dr. D.C. REDDY, Department of Fishery Science and Aquaculture, SV University, Tirupathi Andhra Pradesh, India.

Abstract: This study presents results on Protozoan present in various stages of *Penaeus monodon*. Three categories of protozoans viz .Ciliates sps., Gregarine sps., and Microsporidian sps., were observed in various stages of the host during the year 2011–2012. Ciliates were found in the larvae on the surface of the body, attached to the gills, and appendages. Gregarine sps., were seen in the gut of the larvae. Microsporidian sps., have been noticed in the white faecal matter of the larvae, and white-colored muscle of the host larvae. Larvae are affected by anoxia, starvation, delay of molting, retarded growth rate, zigzag movement, not being attracted towards light, and an ugly, dirty appearance. In cases where these symptoms are severe, infected larvae are dead, as are other microbes. And measured some physical parameters such as Temperature, Salinity, P^H, and dissolved oxygen. As is well known, prevention is better than cure, and most of these have developed in various practices such as lack of water and food quality, increased stocking density, lack of larval management, lack of knowledge in disease diagnosis, inadequate salinity, temperature, P^H, accumulation of organic matter, which have led to the growth of protozoans.

***KEYWORDS*: *Penaeus monodon* larval stages, protozoans, clinical signs, physical parameters, and survival rate.**

INTRODUCTION: Shrimp culture is one of the most important sectors in aquaculture industry that provide balanced diet for a growing society. *Penaeus monodon,* or black tiger shrimp, is one of the most important a species of cultures in India. There are different kinds of shrimp species worldwide, and *Penaeus monodon,* the giant tiger shrimp, is one of the most important species and may be considered the leader in aquaculture. Hatchery producers of tiger shrimp are facing several difficulties during production due to disease outbreaks and poor growth performance (Arrieta *et al*., 2014; Rungrassamee *et al*., 2014). Protozoan parasites are single-celled eukaryotes that live as symbionts, commensals, parasites, and pathogens of crustacea, which are both sessile and mobile forms (Jayakumar et al., 1999). Ciliate protozoans are known to infect crustaceans (Bottom and Ropes, 1988; Abello *et al*., 1990). Obviously these protozoans cause remarkable damage to the Shrimp culture, under the poor management of *Penaeus monodon*.

Penaeus monodon is the host for different protozoan parasites and commensals as ecto and endo-symbionts. These are Acineta, Ephelota gemmipara, Epistylis, Vorticella, and Zoothamnium, Gregarines, Microsporidians, and are considered the major disease-causing agents in shrimp culture. An increase in parasitic infection is supported to affect shrimp farming due to increased stocking density and poor environmental conditions (e.g., ammonium, nitrate, and dissolved oxygen levels) (Kautsky et al., 2000; Gualteros-Rodríguez, 2003). The microbial attack may also alter normal physiological activities such as mobility, breathing, reproduction, and survival rate (Overstreet, 1973).

Some of the protozoans are known to cause disease in weakened larval form in shrimp culture (Parsons & Khan, 1986). Diseases caused by protozoa have been described in the biology of crustaceans. Couch John (1983), and Kruse (1959) gave detailed information about parasites found in commercial shrimp culture. Protozoan parasites are among the most important parasites that affect the growth of natural and cultured shrimp (Overstreet, 1973, 1982, and Lightner, 1983, 1985). Overstreet (1973) made an extensive survey on Zoothamnium infection in Penaeid shrimps and found a positive correlation between the stocking density and the prevalence of infection (Fulks and Main, 1992; Heyward and Hammond, 1990). Most infections are caused by a ecto-symbionts, and Peritrichous ciliates (Overstreet 1973). The Indian tiger shrimp *Penaeus monodon*, faces major problems due to bacterial and protozoan infections. In general protozoan infection causes more damage in the production of shrimp starting from the larval stages to the juvenile, and adult stage. The protozoan that cause infections are categorised into Microsporidian, Ciliate and Gregarine sps.(Lightner,1983). Protozoan infections occur as both ecto and endo-commensals; *Zoothamnium* and *Vorticella* are ecto-commensals whereas *Microsporidian* and *Gregarine* are endo-commensals. Ciliate protozoan infections include *Ciliates, Zoothamnium, Vorticella* and *Acineta* (Jayathi and Bandyapadya, 2011).

Materials and methods:

Observation of Eggs, Larval and Post larval stages

The present work is focused to identify different types of protozoans such as ciliates, microsporidian, and gregarine sps. in eggs, different larval stages, post larval stages of *Penaeus monodon*, and to identify the effect and changes observed in shrimps during different developmental stages. Experiments were performed from September 20, 2011 to September 30, 2012 in Laboratory of Hariharan Shrimp Hatchery, Tupilipalem, Gudur, with help from Technician and Hatchery manager.

A minimum of 50-100 samples were collected from each tank for observation of the microbes. Details of samples, analysis, percentage of prevalence, number of Protozoans Clinical signs, and treatment details are mentioned in table column. During experiment a few pictures were taken by Digital camera through research Microscope. Protozoans were identified based on descriptions of species morphology (Couch 1983, Moravec et al. 1995, Vidal-Martinez et al. 2002, Fernández-Leborans et al. 2006). Characteristics and classification of protozoan referred from An Annotated List of Protozoan Parasites, Hyperparasites, and Commensals of Decapod Crustacea (Sprague and Couch 1971).

Protozoan diseases were observed in larvae and post larvae of *Penaeus monodon*, and the larval samples were collected from each tank using 250 ml beaker and estimated by random sampling method for

larval populations. Sampling was done every month. Clinical symptoms of protozoan diseases like ciliate infections, microsporidia and gregarine. The larvae and post larvae were observed under a research microscope and changes in the physical appearance were recorded.

The behaviour of the larvae, their feeding patterns and activity were also observed. Observations were made on the seasonal prevalence of protozoan parasites, and in relation to mortality and survival. Further, the infected larvae and post larvae were treated with antibiotics and chemicals. The antibiotics such as Chloramphenicol (CP) --1.0 to 3.0 ppm,Formaldehyde-10 to 15 ppm, Treflan (TFL)-0.5 to 1.00 ppm, Oxytetracycline (OTC)-1.0 to 4.0 ppm, EDTA-10 to30 ppm were used to see whether the infections could be controlled.

Fig-1 Map of Andhra Pradesh showing the area visited for field/ Research work

Results:Table-1 presents results on the prevalence and intensity of protozoan parasitic infection in the eggs, larvae and post larva of *Penaeus monodon*. It is cleared from the results that five stages viz, egg, Nauplius-III, Zoea I-III, Mysis I-III, and Post larvae I –X of *Penaeus monodon* have been examined for the presence of Zoothamnium sps. (Z), Acineta sps. (A), Vorticella sps. (V), Epistylis sps.(E), Microsporidians sps. (M) and Gregarines sps. (G).The table also provides data on percentage prevalence of infection by the protozoan parasites, and the location, and clinical symptoms in infected species. Zoea I-III stages showed maximal infection (50%) followed by eggs (36.3%), Mysis I-III stage (35.7%), Post larvae I-X (25%) ,and Nauplius III stage (21.6%). In absolute terms Vorticella sps has been found in large numbers in all the stages examined, followed by Zoothamnium sps. All the infected stages exhibited abnormal clinical symptoms like delayed hatching, delayed moulting, reduced feed consumption, whirling movement, upward jumping, empty gut, brownish/reddish hepatopancreas, white faecal matter etc.

Table-2 provides data on seasonal variations in the physical parameters such as Temperature, Salinity, P^H, and Dissolved Oxygen (DO) of the larval tank water and corresponding variations in the percentage prevalence of protozoan parasites, and the percent survival of Nauplius –III after treatment. The studies were conducted on a monthly basis starting from 25.09.2011 to 20.06.2012 covering different seasons like rainy, autumn, winter, and summer seasons. The results presented also show the percent survival after treatment with antibiotics. Protozoan parasites were found to be significantly higher (percent values) during rainy and winter seasons compared to autumn and summer seasons. The percent survival of Nauplii III after antibiotic treatment was found to be greater than 80.0 during all the seasons.

Table-3 shows the random sampling analysis of Zoea stage of *Penaeus monodon* with regard to stocking density, physical parameters of the larval tank water, percent occurrence of ciliate protozoan parasites and survival percentage before and after antibiotics treatment during different seasons of the year. Maximum stocking density was recorded in the month of September. Greater than 20% parasitic infection was observed in the months of September. (21.7%), March (20.8%), and April (22.2%). Post antibiotic treatment, maximal percent survival was obtained in the months of February (96.7%), and October (95.4%). On the other hand minimal parasitic infection has been recorded in the month of January (15.0%).

Table-4 presents results pertaining to the random sampling analysis of Mysis larvae of Penaeus monodon on the stocking density, physical parameters of the hatchery larval tank water, percentage prevalence of ciliate protozoan parasites and percent survival before and after antibiotic treatment. High stocking density was recorded during the month of November and December. Parasitic infection was found to be 25.6% in March, 27.0% in June and 25.8% in November. Maximum percent survival has been observed in the month of January (91.9%), February (93.0%), and March (92.7%) after antibiotic treatment.

Table-5 clearly shows the random sampling analysis of post larvae of *Penaeus monodon* in relation to stocking density, Physical parameters of the larval tank water, number of ciliate protozoan parasites, and percent occurrence of parasitic infection survival rates before and after treatment during different seasons of the year. Greater stocking density has been recorded in the month of September (0.69 million), February (0.70 million), and March (0.71 million). Maximum numbers of ciliate protozoan parasites were found during April (0.15million), September(0.16 million), March (0.17million), May(0.18million), and June (0.19million). The percent parasitic infection was higher during the months of April (23.4%), March (23.9%), May (26.85%), and June (29.2%). The number of Post larvae survival post infection was 0.59 million in January, and 0.61 million in October. The corresponding percent survival of post larvae after antibiotic treatment was 92.2% and 93.6% respectively. On the other hand minimal parasitic infection was 0.11 million both in October and January. The percent survival was 92.2%, and 93.65% in October and January respectively after antibiotic treatment.

Table-1.Prevalence and intensity of protozoan parasites in the eggs, larvae, and post-larvae stages *Penaeus monodon.*

Stage	No. of Host analysed	No. of infected Hosts	Prevalence (%)	Number of ciliates				Location and Clinical symptoms
				Z	A	V	E	
Egg	110	40	36.3	15	03	20	12	Not hatching; asymmetrical Cleavage in inner part; Blackish color; abnormal development of Nauplii in the egg; Delayed hatching; settled at the bottom..
Nauplius- III	600	130	21.6	10	-	45	10	Delayed moulting; moving away from the light source; fungi attached to lateral side of appendages; Surface of the body looking like a mat and Colony formation.
Zoea I-III	600	300	50	40	05	45	15	Less feed consumption; empty gut, Swimming at the edge of beaker; settling at the bottom; whirling movement; delayed moulting; Very week; difficulty in moulting; Upward jumping; filamentous fungi attached on the basis of 2nd Pleopod, and 5th Pereopod; ventral side near the Mouth parts; surface, lateral side of the gills lamella.
Mysis I-III	700	250	35.7	42	-	55	10	Microsporidia: Empty gut, and white, brown to red color of Hepatopancreas, weak movement; white patch present in the sixth segment, telson, and uropod; White fecal matter. Gregarine: In the Mid gut, spherical shape, and also segmented body with dark color. Fig.1, Fig-2, and Fig-3.
Post larva I-X	800	200	25	51	-	60	18	Gregarine gametes and Microsporidian spores observed.

The prevalence of host infection was calculated by the method of **Margolis et al. (1982):**
Prevalence % = Number of samples infected / Number of samples examined x 100.

Fig-1:Zig – zag movement of the larvae Settled at the bottom

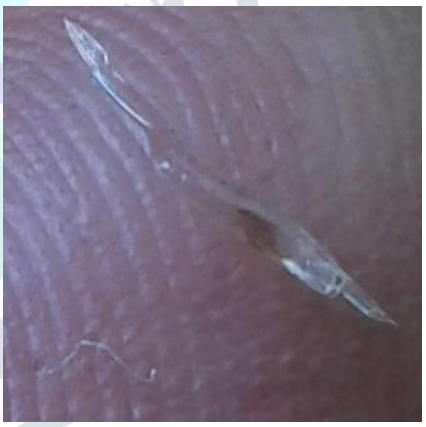

Fig-2:Larva showing white patches ,and faecal matter white in colour

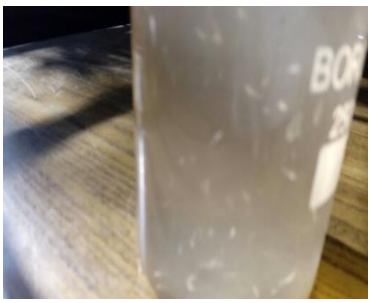

Fig-3: Larvae exhibiting negative photo-taxis, settling pattern at the bottom, and upward movement

Table- 2.Seasonal variations in the physical parameters of the larval tank water, percentage prevalence of Protozoan parasites and percent survival of Nauplii-III larvae of *Penaeus monodon* during 2011-12.

Sl. No	*Date*	Season	Stocking density in millions	Physical parameters				Protozoan parasites		Survival millions	Percentage of survival after treatment
				Temp (0^0C)	Salinity (ppt)	P^H	DO (ppm)	Millions	Prevalence %		
1	25-09-2011	Rainy	1.09	29	30.0	8.0	3.5	0.35	32.11	0.89	81.65
2	22-10-2011	Autumn	1.03	30	29.5	7.9	4.0	0.15	14.56	0.84	81.55
3	20-11-2011	Autumn	1.04	29.5	26.7	7.6	3.2	0.17	16.34	0.90	86.53
4	19-12-2011	Winter	1.10	29.8	28.5	7.7	3.6	0.34	30.9	0.91	82.72
5	15-01-2012	Winter	1.00	30.2	27.8	8.1	2.5	0.23	23.0	0.89	89
6	14-02-2012	Winter	1.01	29	29.4	8.5	2.9	0.21	20.7.	0.88	87.12
7	15-03-2012	Winter	1.06	31	29.8	8.0	2.7	0.17	16.03	0.87	82.07
8	18-04-2012	Summer	1.03	30.5	28.9	7.5	2.8	0.18	17.47	0.92	89.32
9	19-05-2012	Summer	1.02	30.2	28.8	7.7	3.9	0.12	11.76	0.86	84.31
10	20-06-2012	Rainy	1.02	29.5	28.6	7.8	3.2	0.10	9.80	0.84	82.35

The **India Meteorological Department** has also specified **four seasons** in India viz. summer, rainy, autumn, winter. **Rainy season** from **June to September, Autumn** from **October to November, Winter** from **December to February, Summer** from **March to May (**https://en.wikipedia.org/wiki/Climate of India).

The various ecto-commensal ciliates encountered were identified from the standard keys **(kudo, 1966). The prevalence (%)** was calculated from the total number of host examined and the number of infested host with protozoan = Number of sample infested /Stocking density in tank × 100, and **survival %** = Number of host found at the end of experiment/ Number of host introduced (Stocking Density) X 100.

Table-3.Seasonal variations in the physical parameters of the larval tank water, percent occurrence of Protozoan parasites and percent survival of Zoea larvae of *Penaeus monodon* after antibiotic treatment (2011-2012).

Sl. No	*Date*	Season	Stocking Density in millions	Physical parameters				Protozoan parasites		Survival millions	Percentage of survival after treatment
				Temp (0^0C)	Salinity (ppt)	P^H	DO (ppm)	Millions	Prevalence %		
1	25-09-2011	Rainy	0.98	29	30.0	8.0	3.5	0.213	21.7	0.87	88.7
2	22-10-2011	Autumn	0.87	30	29.5	7.9	4.0	0.167	19.19	0.83	95.40
3	20-11-2011	Autumn	0.86	29.5	26.7	7.6	3.2	0.138	16.04	0.78	90.69
4	19-12-2011	Winter	0.95	29.8	28.5	7.7	3.6	0.153	16.10	0.87	91.57
5	15-01-2012	Winter	0.93	30.2	27.8	8.1	2.5	0.140	15.05	0.78	83.87
6	14-02-2012	Winter	0.92	29	29.4	8.5	2.9	0.179	19.45	0.89	96.73
7	15-03-2012	Summer	0.87	31	29.8	8.0	2.7	0.181	20.80	0.76	87.35
8	18-04-2012	Summer	0.89	30.5	28.9	7.5	2.8	0.198	22.24	0.80	89.88
9	19-05-2012	Summer	0.92	30.2	28.8	7.7	3.9	0.171	18.58	0.85	92.39
10	20-06- 2012	Rainy	0.97	29.5	28.6	7.8	3.2	0.156	16.08	0.82	84.53

Table-4. Seasonal variations in Protozoan parasites of *Penaeus monodon*, and percent survival of Mysis larvae between September 2011 and June 2012

Sl. No	*Date*	Season	Stocking density in millions	Physical parameters				Protozoan parasites		Survival millions	Percentage of survival after treatment
				Temp (0^0C)	Salinity (ppt)	P^H	DO (ppm)	Millions	Prevalence %		
1	25-09-2011	Rainy	0.87	29	30.0	8.0	3.5	0.20	22.98	0.79	90.80
2	22-10-2011	Autumn	0.79	30	29.5	7.9	4.0	0.14	17.72	0.68	80.07
3	20-11-2011	Autumn	0.89	29.5	26.7	7.6	3.2	0.23	25.84	0.72	80.89
4	19-12-2011	Winter	0.89	29.8	28.5	7.7	3.6	0.16	17.97	0.80	89.88
5	15-01-2012	Winter	0.86	30.2	27.8	8.1	2.5	0.19	22.09	0.79	91.86
6	14-02-2012	Winter	0.85	29	29.4	8.5	2.9	0.20	23.5	0.79	92.94
7	15-03-2012	Summer	0.82	31	29.8	8.0	2.7	0.21	25.60	0.76	92.68
8	18-04-2012	Summer	0.79	30.5	28.9	7.5	2.8	0.15	18. 98	0.69	87.34
9	19-05-2012	Summer	0.80	30.2	28.8	7.7	3.9	0.18	22.5	0.72	90.0
10	20-06-2012	Rainy	0.78	29.5	28.6	7.8	3.2	0.21	26.9	0.70	89.74

Table - 5. Seasonal variations in Protozoan parasites of *Penaeus monodon*, and percent survival of **Post larvae** between September 2011 and June 2012**.**

Sl. No	*Date*	Season	Stocking density in millions	Physical parameters				Protozoan parasites		Survival millions	Percentage of survival after treatment
				Temp (0^0C)	salinity (ppm)	P^H	DO (ppm)	Millions	Prevalence %		
1	25-09-2011	Rainy	0.69	29	30.0	8.0	3.5	0.16	23.1	0.62	89.85
2	22-10-2011	Autumn	0.66	30	29.5	7.9	4.0	0.11	16.6	0.61	92.24
3	20-11-2011	Autumn	0.67	29.5	26.7	7.6	3.2	0.13	19.4	0.59	88.05
4	19-12-2011	Winter	0.68	29.8	28.5	7.7	3.6	0.12	19.04	0.54	79.41
5	15-01-2012	Winter	0.63	30.2	27.8	8.1	2.5	0.11	17.46	0.59	93.65
6	14-02-2012	Winter	0.70	29	29.4	8.5	2.9	0.12	17.14	0.62	88.57
7	15-03-2012	Summer	0.71	31	29.8	8.0	2.7	0.17	23.94	0.61	85.91
8	18-04-2012	Summer	0.64	30.5	28.9	7.5	2.8	0.15	23.43	0.58	90.62
9	19-05-2012	Summer	0.67	30.2	28.8	7.7	3.9	0.18	26.86	0.59	88.05
10	20-06-2012	Rainy	0.65	29.5	28.6	7.8	3.2	0.19	29.23	0.59	90.76

DISCUSSION: In general the growth and development of any organism depends on the environmental conditions, quality of food and its resistance against pathogens .In this study an attempt was made to assess the effects of protozoan parasites on the growth and development of *Penaeus monodon* larvae in shrimp hatchery system. Table -1 clearly shows the percent prevalence of parasitic infection caused by Zoothamnium sps. (Z), Acineta sps.(A), Vorticella sps.(V),Epistylis sps.(E), in different larval stages of *Penaeus monodon*. Maximum infection was observed in Zoea stages (50%) and minimum infection in Nauplius stage (21.6%). It is also clear that all the infected stages exhibited abnormal clinical symptoms as presented in Table-1.

As microbial infection greatly reduces Shrimp yield several studies have been conducted to understand this problem in different geographical regions of India (Bower et. al. 1994; Prasadan and Janardhan, 2001; John et. al. 2006). Results obtained in this study corroborate the evidence already presented .Data presented in Table 2 to 5 clearly show the seasonal variations in the physical parameters of the Hatchery larval and post larval tank water, and percent survival of Nauplii, Zoea, Mysis and Post larvae between September 2011 and June 2012. *Penaeus monodon* eggs are small, spherical, dark greenish in color and the size varies from 0.25 to 027 mm in diameter. The eggs usually hatch with in a time line of 12- 17 hours after spawning at normal temperature (28-32^{o}C) which was not seen in infected eggs. The developing Nauplius almost fills the entire space in the egg and undergoes cleavage. Detailed microscopic observation revealed delayed hatching, and a few eggs not undergoing cleavage. The presence of asymmetrical shape and blackish colour clearly shows infection in eggs. The ciliates found to be attached to the surface would possibly result in abnormal shape and hatching of eggs (Aquaculture Extension Manual no.19 May 1996).

Zoothamnium sps. are ecto-commensals that bind to the surface of the host, and thus the infection does not lead to mortality (Mahasri et. al., 2018). In this study, infection has been seen on the surface of the body and gills; however, Zoothamnium infection of gills causes mortality because it leads to difficulty in gaseous exchange. Usually under normal dissolved oxygen concentrations mortality may not occur whereas when the DO levels drop below 2.6 ppm mortality may occur. Obviously the results of this study are in concurrence with previous studies (Sterling et. al., 1964).Tonguthai (1991) reported that during summer DO levels decrease to a great extent due to increased salinity and hence the infected species suffer from

inadequate DO levels, breath hard, movement becomes slow and sluggish resulting in difficulty finding food. As a result moulting pattern is disrupted leading to stunted growth and decrease in economic value. Apparently Zoothamnium infected shrimp try to swim towards the surface waters/shore for better DO supply facilitating investigation of white matter over the surface of the organism. Microscopic examination of white matter revealed that the parasite found is Vorticella which affect the growth of the larvae and Post larvae by inhibiting the feeding activity.(Patoka et al., 2016; Shailender et al., 2012).Crustacean shell disease caused by Epistylis sps.has been recorded in lernaeid (Copepod) crustaceans (Silva –Souza and Rosin, 2005; Abdallah, et. al., 2011). However results of this study show lower rate of Epistylis infection compared to that of Zoothamnium and Vorticella.

Previous studies have reported that epibionts cause damages to the host in multiple ways such as decreased survival, fecundity, lesions and diseases, disturbance of location, decreased competitive ability, increased susceptibility to predation, increased energy demands, and faster sinking rates (Bozkurt and Genc, 2009). In this study, it was found that Acineta infection is very less or negligible compared to that of all other parasites detected after the treatment probably because the treatment procedure might have cleared the acineta infection. Less prevalence of Acineta infection reported by Kakoolaki and Afsharnasab (2016) are in agreement with the data presented in this study. Obviously the best method to prevent protozoan infection is to maintain healthy farming practices in the hatcheries such as timely change of water which may prevent ciliate protozoan parasite infection. Formalin is one of the strong agents that enables the recovery of shrimp. The role of formalin in preventing parasitic infection in hatcheries has been well established. Chloramphenicol, Treflan and Oxytetracycline have also been found to protect the shrimp during parasite infection. It has been reported that these antibiotics are capable of killing the pathogens. The results of this study clearly indicate that the treatment of the infected of larvae with chemicals /antibiotics resulted in appreciable recovery in all the larval stages. Sterling and his co-workers (1964) demonstrated that protozoan infection could be controlled by treating with formaldehyde. In the present study the recovery of protozoan infection has been achieved by synergetic effect of formalin and other chemical compounds.

References:

1. Abdallah, VD; Azevedo, R K. and Silva, R J., 2011. “First record of an epibiont protozoan Epistylis sp. (Ciliophora, Peritrichia) attached to Amplexibranchius bryconis Thatcher & Paredes, 1985 (Copepoda, Ergasilidae) from Peixe’s River, state of São Paulo, Brazil”. Crustacean, vol. 84, no. 9, p. 1139-1144.
2. Abello, P., Macpherson, E., 1992 “.Epibiosis and rhizocephalan infestation patterns in relation to the reproductive biology Lithodes ferox Filhol, 1885 (Anomura: Lithodidae)”. Journal of. Crust. Biol. 12, 561–570.
3. Arrieta, M. C., Stiemsma, L. T., Amenyogbe, N., Brown, E. M. & Finlay, B. “The intestinal microbiome in early life: health and disease. Front. Immunol.5, 427–427, https://doi.org/10.3389/fimmu.2014.00427 (2014).

4. Bottom. M.L., Ropes, J.W., 1988. "An indirect method for estimating longevity of the horseshoe crab (*Limulus polyphemus*) based on epifaunal slipper shells (Crepidula fornicata)". J. Shellfish Res. 7, 407–412.

5. Bozkurt, A. and Genc, E., 2009. "First record of the epibiont protozoan *Epistylis sp.* on zooplankton (Copepoda, Cladocera, and Rotifera) from the Asia River, Turkey". Crustaceana, vol. 82, no. 5, p. 515-530.

6. Couch John, A. 1983, "Diseases caused by protozoa in the biology of crustacean", edited by proven zano A.J., editor in chief Blise. D.E. Vol. 6, pp. 79-111.

7. Fulks, W., and Main, K.L. (1992) Diseases of Cultured Penaeid Shrimp in Asia and the United States. Proceedings of a workshop in Honolulu, Hawaii.The Oceanic Institute.

8. Gualteros-Rodríguez, W. 2003, "Estudio de la comunidad de nemátodosmeiobentónicos en 2 estanques de producción de camarón. Golfo de Guayaquil, Ecuador. MS thesis. Escuela Superior Politécnica del Litoral, Facultad de IngenieríaMarítima y Ciencias del Mar. Guayaquil, Ecuador

9. Heyward, A.J., and Hammond, L.S. (1990). "Biotechnology for Aquaculture". INFOFISH International

10. Jayati Chakraborti, P. Bandyapadhyay K. (2011)."Seasonal incidence of protozoan parasites of the black tiger shrimp (*Penaeus monodon*) of Sundarbans", West Bengal, India. *J Parasit Dis* (Jan-June 2011) 35(1):61–65.

11. Jayasree L, Janakiram P, Madhavi R 2001. "Epibionts and parasites of *Macrobrachium rosenbergii*, and *Metapenaeus dobsoni* from Gosthani estuary". J Nat Hist 35:157–167.

12. Kakoolaki, S; and M Afsharnasab. 2016. "Prevalence and intensity of protozoan ecto-parasite of the *white leg shrimp* (*Penaeus indicus*) in Helleh site, South of Iran". Iranian Journal of Aquatic Animal Health. 2(1); 17-23.

13. Kautsky. N. P. Rönnbäck, M. Tedengren, M. Troell, 2000 "Ecosystem perspectives on management of diseases in shrimp pond farming". Aquaculture. 191:145–161.

14. Kruse, D.N. 1959, "Parasites of the commercial shrimps. P*enaeus aztecusies. P. duorarum.* Burken-road and *P. satiferus (Linnaeus)*". Tulane Stud. Zool. 7 :123-44.

15. Lightner, V. 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.

16. Overstreet, R.M. 1973, "Parasites of some Penaeid shrimps with emphasis on reared hosts". Aquaculture. 2: 105-140.

17. Overstreet, R.M. 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.

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

19. Patoka Jiri, Martin Blaha, Miloslav Devetter, Katerina Rylkova. Zuzana Cadkova´.Lukas Kalous,2015. ” Aquarium hitchhikers: attached commensals imported with freshwater shrimps via the pet trade”. Spring, Biol. Invasions DOI 10.1007/s10530-015-1018-9
20. Rungrassamee, W. et al. 2014 “Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*)”. PLoS ONE, Volume 9, issue3: e91853. https://doi.org/10.1371/journal.pone.0091853 (2014).
21. Shailender M, Krishna P V, Suresh Babu CH, 2012 “Factors affecting the post larval production of *Macrobrachium rosenbergii* (de man) by using different types of feeds”. International Journal of Bioassays (IJB), pp-265-268.
22. Silva-Souza, AT. And Rosim, DF., 2005.”Parasitismo de peixes do córrego do Feijão, São Carlos, São Paulo, Brasilpor crustáceo lerneideo emepibiose com Epistylis sp. (Ciliophora: Peritrichia: Sessilida)”. Parasitologia Latino Americana, vol. 60, p. 349.
23. Sterling K. Johnson, Jack C. Parker, and Hoyt W. Holcomb. 1964. “Control of Zoothamnium sp. on *Penaeid shrimp”*. World Mariculture Society Workshop. Page no 321-331.