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Research Article

STUDY OF MICROSPORIDIOSIS IN MYSIS III AND POST LARVA STAGES OF *PENAEUS MONODON*

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ABSTRACT

Microsporidiosis is a significant protozoan disease affecting the larval and post-larval stages of *Penaeus monodon*, contributing to substantial economic losses in shrimp hatcheries. The present study investigates the prevalence, clinical manifestations, and environmental associations of microsporidian infections in mysis III and post-larval stages of *P. monodon* collected from Gudur, and Nizampatnam regions of Andhra Pradesh. Through lens-hand inspection, direct microscopy, and squash preparations, microsporidians were identified in the gut, hepatopancreas, muscle, and uropod, often accompanied by ciliates co-infections (*Epistylis*, *Vorticella*) and fungal attachments. Infected larvae exhibited distinct clinical signs, including white gut, white fecal threads, hepatopancreatic discoloration, reduced motility, growth retardation, and reverse swimming behavior. Environmental parameters demonstrated seasonal variability, with higher infection prevalence recorded during the summer months: 40.9% in mysis III (110 examined; 45 infected) and 36% in post-larvae (100 examined; 36 infected). Winter prevalence was moderately lower at 31.6% and 35%, respectively. Elevated water temperature, increased salinity, and reduced dissolved oxygen coincided with higher disease incidence, underscoring the role of abiotic stress in pathogen proliferation. The findings emphasize that microsporidians act as commensals, opportunistic pathogens, or severe parasites depending on infection intensity and host condition. The study reinforces the critical need for routine hatchery monitoring, improved diagnostic capability, and better-trained technicians to ensure optimal larval health. Maintaining stable physico-chemical parameters, regulating stocking densities, and adopting bio-secure feeding practices remain essential strategies to mitigate microsporidian outbreaks. This work contributes to the growing understanding of protozoan infections in penaeid aquaculture and highlights the necessity for integrated health management in shrimp hatcheries as well as pond culture farmers.

Keywords: *Penaeus monodon*, Protozoan parasites, Ciliates, Clinical symptoms, Physical parameters.

INTRODUCTION

In shrimp farming, disease is considered the primary factor contributing to low yield (Kumari *et al*., 2018; Asche *et al*., 2020). According to Kumari *et al*. (2018), pathogens, nutritional deficiencies, and environmental fluctuations are the main causative factors for disease manifestation in shrimp culture. At present, shrimp aquaculture represents a multi-million-dollar global industry. Microsporidians, belonging to the phylum *Microspora*, class *Microsporea*, and order *Microsporida*, are obligate intracellular endoparasites that infect a wide range of shrimp and prawn species (Johny *et al*., 2006). These parasites are capable of infecting a broad spectrum of invertebrate and vertebrate hosts (Didier *et al*., 2000), with nearly half of the known species utilizing insects as their primary hosts (Becnel and Andreadis, 1999). In crustaceans, microsporidians can infect various tissues including the gut, hepatopancreas, muscle, reproductive, and nervous tissues (Walker and Hinsch, 1972; Becnel and Andreadis, 1999; Solter and Becnel, 2000). Although no clear seasonal trend has been established for microsporidian prevalence in crustaceans (Childers *et al*., 1996), Chakraborti and Bandyapadhya (2011) reported microsporidian parasites (0.5–1.5 µm in diameter) isolated from the muscles and hepatopancreas of the decapod crustacean *Penaeus monodon*. Interestingly, infections were observed only during summer and were

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completely absent during spring and autumn. Given the economic importance of shrimp aquaculture and the impact of parasitic infections on yield, it is crucial to assess penaeid shrimp as potential hosts for *Microsporida*. In this context, the present study was undertaken with the following objectives are to collect and identify microsporidian from the samples of mysis III and post-larval stages of *Penaeus monodon*. And to study the effect of physico-chemical parameters and seasonal incidence of microsporidian and ciliate protozoan parasites. For this purpose, shrimp samples representing various developmental stages of *P. monodon* were collected from two selected locations such as Gudur and Bapatla districts for the identification and study of microsporidian protozoan parasites.

MATERIALS AND METHODS

Sample collections

Endo-parasitic protozoa, microsporidian species, were observed in the mysis III and post-larval stages of *Penaeus monodon*. These parasites commonly occur in the digestive tract, particularly in the mid and hindgut as well as in the muscle and hepatopancreas. In this study, microsporidians were collected from the hatchery tanks and ponds containing mysis III and post-larval stages of *P. monodon* and find out to their characteristics and effects on the host. Samples were collected from shrimp hatchery and grow-out ponds located in Nizampatnam to study the influence of physico-chemical parameters on the seasonal incidence of microsporidian species in mysis III and post-larval stages of *P. monodon*. All larvae are transparent so can see internal and external morphology and clinical features.

Microscopic Observation and Examination

The observation of samples was conducted and following three process namely lens-hand observation, direct examination, and the squash (wet-mount) method.

Squash (Wet Mount) Preparation

This method served as the primary approach for the initial microscopic examination. For internal morphological analysis, tissues such as the hepatopancreas, muscle, and gills from live, infected specimens were carefully dissected, placed on a glass slide, gently squashed, and examined under a light microscope. For post-larva samples, the entire specimen was squashed and examined microscopically to observe tissue abnormalities and the presence of pathogens.

Clinical and Morphological Observations

Infected mysis III and post-larvae stages of *P. monodon* were collected from the Gudur hatchery and culture ponds in Nizampatnam, Andhra Pradesh, were examined microscopically for clinical signs of infection. Observable external and internal symptoms included a white gut with gaps, white fecal matter, white patches, and a brick-red colored hepatopancreas. Infected mysis III and post-larva exhibited mixed symptoms, such as white feces appearing as thread-like structures (Figure 1). Ciliates of *Zoothamnium* in the telotroch stage appeared as small white dots on the surface of the host and *Vorticella* and *Epistylis* were attached to the edge of appendages. Additionally, mysis III and post-larva stages of *P. monodon* showed signs of microsporidiosis, along with fungal attachments on the appendages, as observed directly with the naked eye and through a hand-held lens following traditional observation methods (Table -1).

RESULTS AND DISCUSSION

The results clearly show that protozoan microsporidians occur as symbiotic commensal, pathogens (may damage physiological functions of the host) and parasites (may or may not be pathogens that cause disease, but have the potential to produce a negative effect on the host, especially during heavy infections). Table-1 presents results on external clinical symptoms in Mysis III of *P. monodon* (Figure 1), include all white in colour of abdominal segment, telson, and foregut; hepatopancreas brown red in colour, and empty gut. Data on environmental variables like temperature (29.20C), Salinity (29.8 ppt), PH (8.5) and dissolved oxygen (2.9 mg/lit) have also been recorded during winter season. During summer season, the water temperature recorded was (30.20C), salinity (30.4 ppt), PH (8.0) and dissolved oxygen concentrations were (2.5 mg/lit) where as in winter season. So the environmental parameters varied considerably between summer and winter seasons. The results also show the number of mysis-III observed, number of mysis-III infected, and percentage prevalence of infection. In the summer season, 45 were infected with microsporidians out of 110, and the percentage prevalence was 40.9%. But in the winter season out of 120 observed 38 were infected with microsporidians, and the percentage prevalence was 31.6% (Table- 1). Table-2 presents and results on external and internal symptoms of infected post larval stage of *P. monodon*, and percentage prevalence of infection during winter and summer seasons. The infected post larvae showed whitish abdominal segment, and telson, a white line from the tail where it bends at the sixth abdominal segment, fore gut, whitish lateral side of the mid gut, size variables of homogeneity and heterogeneity, brownish red hepatopancreas, slow heartbeat, empty gut, reverse swimming , whitish muscle and lateral side of the gut (Figure 2).Some ciliates viz. epistylis and vorticella were attached to the external parts of the microsporidian infected of host such as surface, mouth and abdominal parts (Figure3). The results also show the number of post larvae observed, number of post larvae infected, and percentage prevalence of infection. In the summer season, 36 were infected with microsporidians out of 100, and the percentage prevalence was 36%. But, in the winter season out of 140 observed 49 were infected with microsporidians, and the percentage prevalence was 35% (Table-2).

Protozoan parasites, pathogens and commensals in and outside of the host's body. Tissues infected by microsporidian parasites include striated and smooth muscle, and the gonads. Couch, (1978) observed that the percent infection in penaeid culture ponds usually reach 10% in certain conditions. Apparently, severe infections in cultured penaeids may cause chronic disease and mortality (Couch, 1978; Lightner, 1983), and parasitic castration (Enriquez *et al*., 1980) leading to production of unmarketable products. Protozoans display an amazing variety of inter-specific relationship among which parasitism is the most common and well documented in the class Microsporea of the phylum Microspora. The microsporidians are one of the most important group of protists which has unicellular spores. Microsporidians were infected more in the summer season 2012 (40.9%) than in the winter season 2012 (31.6%). This was noticed in the mysis III stage of *P. monodon,* was noticed the environmental variables viz. Temperature 30.2^{0}C, salinity 30.4ppt, PH 8.0, and DO 2.5 mg/lit during summer season, but temperature 29.20C, salinity 29.8 ppt, PH 8.5, and DO 2.9 mg/lit during winter season. And in PL of *P. monodon*, microsporidians were affected in the summer season (36%). And measured the environmental variables viz. Temperature 31.2^{0}C, salinity 30ppt, P^H 8.5, and DO 3.5 mg/lit during summer season but temperature 30.2^{0}C, salinity 31 ppt, P^H 8.0, and DO 3.2 mg/lit in winter season. Microsporidiosis disease along with co-infection and interrelation with ciliates and fungi (thread) in mysis III and Post larva stages of *P. monodon* noticed through compound microscope.

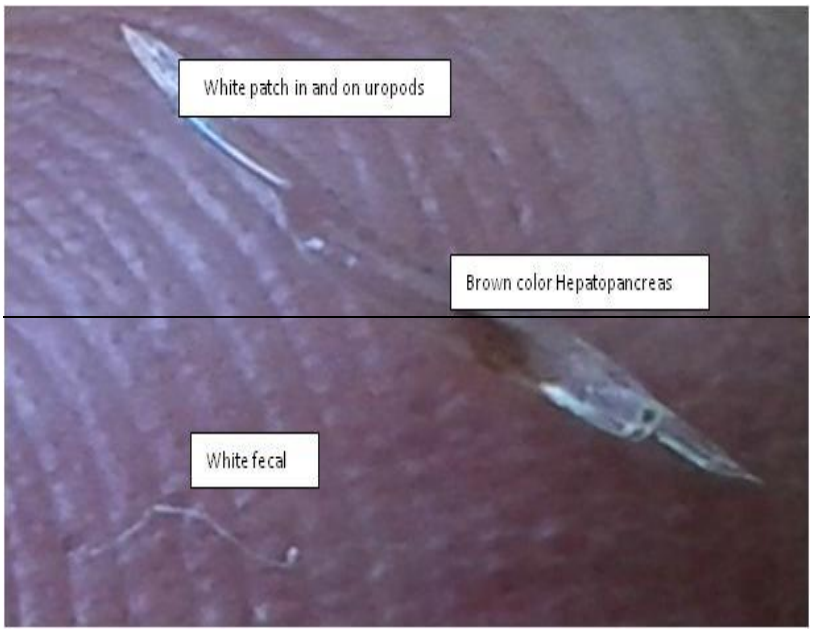

Figure 1. Microsporidiosis symptoms showing in Mysis-III of *P. monodon,* white gut and fecal matter white in colour.

Table 1. Stage, location with date, season water analysed and external and internal clinical symptoms of microsporidiosis, number, infected and calculated the infection in the infected host of *P. monodon.*

Stage	Dates and season	Environmental parameters	Observed the external and internal symptoms of the infected host	Number of larvae observed	Number of larvae infected	Percent infected
Mysis-III	13th May 2012 Nizampatnam During summer Season	Temperature-30.20C, Salinity– 30.4 ppt, PH-8 and Dissolved oxygen-2.5 mg/lit, and water is brown and dirty in color.	Slowly moving of food particles in the gut, peristalsis movement is very slow, observed the white patches on uropod, white color in fore and mid gut, red brown color of hepatopancreas,	110	45	40.9%
	10th January 2012	Temperature-29.20C, Salinity– 29.8 ppt,	White in colour of abdominal segments and	120	38	31.6%

Nizampatnam During Winter Season	PH-8.5 and Dissolved oxygen-2.9 mg/lit, and water is brown and dirty in colour.	size variables.

Figure 2. Post larvae of *P. monodon* showing whitish abdominal segments, gut and uropod and size variables.

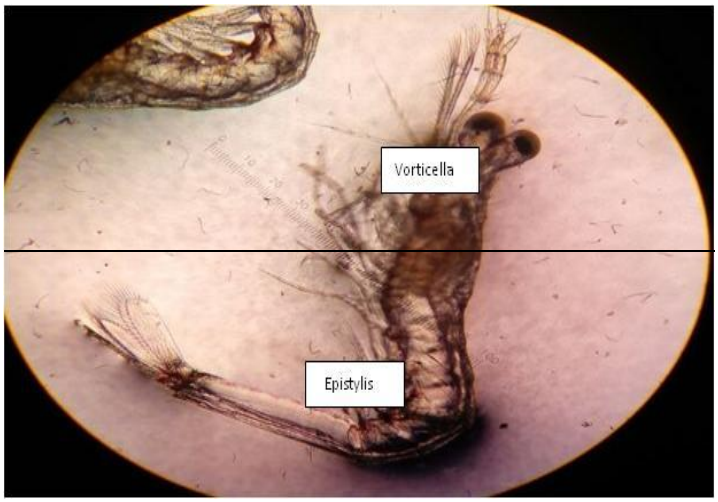

Figure 3. Epistylis and vorticella sps., attached to the lateral, and ventral side of sixth abdominal segment and mouth parts of the post larva stage along with fungi (10 X).

Table 2. Stage, location with dates, season, water analysed and external and internal symptoms of microsporidiosis of the infected host of *P. monodon*.

Stage	Dates	Environmental parameters	External Symptoms of microsporidiosis	Season	Number of Post larvae observed	Infected of the Post larvae	Percent infected
		Temperature-31.20C, Salinity –	Growth retardation, abdominal segments				

Post larvae stages	13th May 2012 and10th January 2012 from Nizampatnam during summer, and winter season.	30 ppt, PH-8.5 and Dissolved oxygen-3.5 mg/lit, and water is brown and dirty in colour.	white in colour, Slowly movement, and few larvae are swimming in reverse.	Summer	100	36	36%
		Temperature-30.20C, Salinity – 31 ppt, PH-8.0 and dissolved oxygen-3.2 mg/lit, and water is dirty in colour.	White patches on the telson, white colour of abdominal segments and white colour of digestive tract	Winter	140	49	35%

Water quality parameters play a vital role in hatchery as well as culture farms because these factors effect on growth and development of the host. Maintenance of good water quality is essential for optimum growth and survival of shrimp. The successful establishment of a species in a given habitat depends on the ability of each of its developing stages to adapt to the existing environment (Charmantier,1998). Salinity, and temperature are the most important abiotic factors affecting growth and survival of aquatic organisms (Kinne, 1963, 1964). While early stages of development are the most sensitive phases in the complex life cycle of marine invertebrates and the larvae should be reared close to optimal conditions to maximise survival. The optimal environmental conditions for growth are species specific and differ between life-history stage and season (Costlow *et al*., 1960; Bas and Spivak, 2000). During the nauplius stage, the rate of development is mainly influenced by abiotic factors, as the nauplii do not feed. The most important water quality parameters to be monitored during larval stages are temperature (30± 2°C), salinity (32 ± 2 ‰), and pH (7.8-8.3) (Liao 1986, Body and Liao 1987). Temperature is probably the most important environmental variable in shrimp hatcheries and cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature effects on the metabolism and immune system of shrimp. The optimum temperature range recommended for rearing penaeid shrimp is 28-30°C (Licop,1988).

Good water quality is characterized by adequate oxygen and limited level of metabolites. Excess feed, faecal matter and metabolites usually exert tremendous influence on the water quality in larval tanks. Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Hence, Oxygen affects the solubility and availability of many nutrients. Low level of dissolved oxygen can cause damage in such a way that substances could be converted from oxidized to the reduced form. Decrease of dissolved oxygen can be directly harmful to shrimp and cause a substantial increase in the level of toxic metabolites reducing growth and moulting and increasing mortality.P^H is one of the vital environmental characteristics, which decides the survival and growth of shrimp under culture and also affects the metabolism and other physiological process of the host. The optimum range of P^H to be maintained for maximum growth and production is pH 6.8 to 8.7. Water transparency is another factor that influences growth and survival because muddy water hinders to penetration of light reducing oxygen availability. Therefore, periodically exchange of water required. Salinity influences food consumption, conversion efficiency and, hence, growth and survival of cultured shrimp (Venkataramaiah *et al.,* 1972). There are wide variety of reports regarding salinity tolerance of different penaeid shrimp. Though Motoh (1981) reported that *P. monodon* could withstand salinity ranges of 0-60 ppt, 15-25 ppt salinities are considered ideal for better growth of *P. monodon* grow-out (Boyd, 1989). Poor growth rate and high mortality of *P. monodon* juveniles were noticed at salinities lower than 10 ppt (Cawthorne *et al.,* 1983). At high salinity the larval and post larval stages grow slowly but are healthy and resistant to diseases. In case the salinity is low in winter and rainy season the shell will become weak and exposed to diseases. The swimming activity of the larvae changes dramatically, Nauplii swims surface of the water, Zoea stage swim rapidly and forwards, usually in circles, Mysis swim backwards with intermittent flicks of their tails, maintaining themselves in the water column PL swim rapidly and consistently forward, searching for food whilst being maintained in the water column by strong aeration. Generally, the more larvae swimming actively, the better their quality. Most of the shrimp hatcheries apply specific management practices to prevent the outbreak of diseases. If infected mysis larva and post larva has zig-zag movement, slowly locomotion on the edges of the beaker.

CONCLUSION

Microsporidians are located in the muscle, gut, and uropods, where they grow and develop, causing the affected tissues to appear white or milkish in color. Ciliates were also attached to the pereopods, appearing like thread-like structures on the appendages, resulting in growth retardation. Infection of the host with microsporidian species occurs in the gut, hepatopancreas, and muscle. The infected areas become white in color, vary in size, and release whitish fecal matter. At present, loss in production has been attributed to the effects of microbes, environmental changes, poor water quality, high stocking

density, poor hatchery management, use of raw food materials such as crab, oyster, Artemia, and polychaetes, lack of constant monitoring and diagnostics, and inadequately qualified staff working as section technicians. Regular monitoring of water quality parameters, feed densities, larval development, and the presence or absence of diseases in larval culture tanks comprises the day-to-day observations in the hatchery. Many technicians working in hatcheries today are not properly trained or have not undergone sufficient training. It is suggested that these technicians should undertake thorough training to understand the basics of larval rearing technology and establish constant contact with training institutions or fellow technicians to gain insights into new management techniques in the hatchery industry.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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