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Impact of Diseases on the Growth and Survival of Giant Freshwater Prawn, *Macrobrachium rosenbergii* (De Man) Larvae in the Hatchery Level

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Abstract: The present study was conducted, in the freshwater hatchery, Kakati Aqua Tech Ltd. and pathological laboratory in the Department of Zoology and Aquaculture, Acharya Nagarajuna University, Andhra Pradesh, India, to know the prevalence and controlling measures of bacterial, fungal and protozoan diseases of larval and post larval stages of *M. rosenbergii* in commercial scampi hatcheries. A variety of pathogens are found in larval, juvenile and adult *Macrobrachium rosenbergii*, including fouling protozoans such as *Epistylis, Zoothamnium* and *Vorticella*; and pathogenic bacteria such as *Vibrio, Aeromonas, Pseudomonas, Edwardsiella* found during the life cycle. Protozoa, bacteria and fungi are the main important diseases have found in the larval phase and their impact is more on the survival rate of post larvae. This work is to review various pathogens at different life stages and survival of post larvae of giant freshwater prawns.

Key words: *Macrobrachium rosenbergii* % Diseases % Survival

INTRODUCTION

Freshwater prawn culture is rapidly expanding and is an important species cultured in many countries. Production of healthy and quality seeds has been a major obstacle in the expansion of scampi hatcheries. The production cycle depends on disease causing agents, environmental factors and sustainable management practices influence in the success of the hatchery operations. The important disease causative agents of infectious diseases in scampi are viruses, bacteria, fungi, protozoans and other parasites. The occurrence of viral and bacterial effect has been reported by Anderson *et al.* [1], Tung *et al.* [2], (Kennedy *et al.* [3], Lightner [4] and fungal diseases by New, [5], Sahul hamid, [6], Sarathi *et al*. [7]. Tonguthai, [8] and Sharshar, [9] reported that serious mortalities in *M. rosenbergii* hatcheries caused by Luminescent bacterial disease due to *Vibrio* spp. The pathogen can enter the hatchery system by various ways, most important through feed, broodstock, instruments, water and unhygienic handlings of workers. According to Olafsen, [10], Phatararpekar *et al.* [11] a pathogen associated with hatchery systems because the host-micro interaction fades for reaching implication on larval health development and out breaks of disease. In the hatchery systems, the pathogen associated with prawn eggs and larvae may be different due to the various water resources, artificial feeds and higher density of organisms. Some of the pathogens may be opportunistic pathogen causing disease in stressed larval populations.

In the present study was conducted in the freshwater hatchery, Kakati Aqua Tech Ltd. and pathological laboratory in the Department of Zoology and Aquaculture, Acharya Nagarajuna University, Andhra Pradesh, India.

MATERIALS AND METHODS

The study was carried out in a commercial hatchery (Kakati Aqua Tech Ltd) near Vijayawada; Andhra Pradesh, India (Figs. 1, 2) where the clear water methods was followed. The larval rearing tanks (each tank stocking density is 100 larvae per/L) were selected randomly and were monitored for the prevalence of bacterial, fungal and protozoan's diseases through the entire production cycles carried out in the year 2010. Broodstocks were collected from wild and pond reared was used for hatching. Larvae collected from hatching tanks and transferred into different larval rearing tanks. The all stages of larvae (zoea I to X) are corresponding to each

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Fig. 1: Location of the freshwater prawn Hatchery, Kakati Aqua Tech Ltd.

Fig. 2: Location of the freshwater prawn Hatchery (More Closer), Kakati Aqua tech Ltd.

batch was sampled throughout the developmental stages including post larvae. The usual times of one larval cycle ranges from 22-27 days, duplicate samples of the various developmental stages of the larvae collected from every 2-3 days and observed under microscopically and check the status and analyses for microscopically status of samples were collected ad preserved in sterilized bottles. *Artemia salina* is used for live feed for the *M. rosenbergii* larvae and egg custard used as a supplementary food for advanced larvae (VI stage onwards).

Microbial disease has been reported to be a major limiting factor in production both in hatchery and cultured prawns of *M. rosenbergii*. Most of bacteria known from the scampi are common epibionts, which appeared to cause little harm. A number of ciliate protozoan occur as symbionts, commensal, parasites and pathogens of crustacean larvae. The exoskeleton of the scampi larvae served as a substratum on which both sessile and mobile forms of peritrichous ciliates lived and reproduced. Most peritrichous ciliates viz. *Zoothamnium*, *Epistylis*, *Vorticella*, *Lagnophyrus* and *Aceneta* found to attach them on the cuticle, causing mortalities of larvae and post larvae.

RESULTS

In the present study, occurrence and pathological signs of bacterial diseases due to filamentous, non-motile gram negative bacterium, *Lucothrix* sp. and luminescence bacteria of *Vibrio* spp. were observed on zoea and post larval stages. The following pathological effects were observed in case of filamentous bacterial diseases

- Presence of fine, colorless thread like growth on the body surface and gills observed under microscope
- In Zoea larvae and post larvae, filamentous growth on appendages and body surface, interfering with normal locomotory process and with moulting, entrapping of micro organisms causing multiple infections was also noticed in this type of infection.

Due to the infection caused by luminous bacteria, the following gross signs were observed:

- Larvae became weak and opaque, infected larvae exhibited a continuo's greenish luminescence when observed under total darkness.
- Chronic infection resulted in mortalities of larvae and post larvae in large numbers.

Site of Occurrence and Pathological Signs of Ciliate (Protozoan) Diseases: The peritrichous ciliates viz *Zoothamnium* species, *Vorticella* spp. and *Epistylis* spp. were recorded in *M. rosenbergii* larvae and post larvae. All the peritrichous ciliates were commonly noticed on the extended part of the body, on the cuticle and gills. Reddish to brownish gills were observed in case of chronic protozoan infection. 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 mouthparts, hindering the free movement of the mouth parts 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.

Site of Occurrence and Pathological Signs of Fungal Diseases: The fungal diseases of larvae and post larvae were also observed in the present findings. The fungal diseases of larvae are commonly referred to as larval mycosis. The following gross sign have been observed in the present studies. Larvae and post larvae appeared whitish became week and eventually die. Microscopic observations revealed the following symptoms. The fungal hyphae replaced the internal tissues of the prawn larvae and extend outside the prawn body to form discharge tubes and larvae exhibited respiratory difficulties. Fungal hyphae were also observed on the cuticle of the larvae and post larvae. Rapid spreading of the disease was observed in the rearing tanks.

Stage: Zoea (1-5): Percentage of infected larvae and survival rate after infection due to filamentous bacteria (FB), Luminescence bacteria (LB), fungal disease and protozoan diseases and are presented in Table 1. It is evident from the table that protozoan disease has been observed throughout the year, highest percentage of infected 22% zoea was noticed in the month of April and lowest of 15% observed in January during the year, 2010. The high survival rate of 83.7%, 83.4%, 83.87% and 83.13% was recorded during monsoon period in the year 2010 after treatment.

Infection due to filamentous bacteria was occasionally seen. It was found to be prevalent in the month of March, April and May (5%, 11% and 15%) in 2010. Luminescence bacteria were observed to be more prevalent in all the months of year. The occurrence of fungal disease is rare, but 6% infection was noticed in the month of May, 2010 (Table 1).

Stage: Zoea (5 to 10): Advanced larva was also affected with protozoan diseases especially with the ciliate infection. The highest percentage of protozoan diseases in zoea five to ten was recorded as 21% and 20% in

Table 1: *M. rosenbergii*-Types of infections encountered and survival of zoea (1-5) larval percentage in 2010

		Infection									
				Bacterial infection						% of	
	Stocking	Protozoan infection		Filamentous Bacteria		Luminescence Bacteria		Fungal Disease		Survival	
	density										
Months	(Million)	Millions	%	Millions	%	Million	%	Million	%	Millions	%
Jan.	0.09	0.14	14.7	-	-	-	-	-	-	0.82	83.8
Feb.	0.93	0.12	18.2	-	-	-	-	-	-	0.76	81.5
Mar.	0.87	0.17	19.8	-	-	-	-	-	-	0.70	80.6
April.	0.96	0.21	22.5	-	-	-	-	-	-	0.76	79.4
May.	0.88	0.16	18.3	-	-	0.05	5.67	-	-	0.69	78.5
June.	0.97	0.12	12.9	-	-	-	-	-	-	0.81	83.7
July.	0.86	0.14	15.6	-	-	-	-	0.06	6.6	0.74	83.4
Aug.	0.87	0.18	20.6	-	-	-	-	-	-	0.73	84.1
Sep.	0.96	0.17	17.4	-	-	-	-	-	-	0.79	82.6
Oct.	-	-	-	-	-	-	-	-	-	-	-
Nov.	-	-	-	-	-	-	-	-	-	-	-
Dec.	0.85	0.14	16.9	-	-	-	-	-	-	0.71	84.1

Table 2: *M. rosenbergii*-Types of infections encountered and survival of zoea (5-10) larval percentage in 2010

		Infection									
				Bacterial infection						% of	
	Stocking density	Protozoan infection		Filamentous Bacteria		Luminescence Bacteria		Fungal Disease		Survival	
Months	(Million)	Millions	%	Millions	%	Million	%	Million	%	Millions	%
Jan.	0.82	0.14	17.5	-	-	-	-	0.39	4.84	0.71	86.65
Feb.	0.76	0.16	21.7	0.08	10.15	-	-	0.04	5.72	0.67	87.55
Mar.	0.70	0.09	13.7	0.11	15.26	0.09	12.65	-	-	0.60	84.87
April.	0.76	0.14	18.6	0.09	12.19	0.11	14.74	-	-	0.64	83.88
May.	0.68	0.08	12.4	-	-	0.13	19.53	-	-	0.56	81.53
June.	0.80	0.16	20.7	0.14	16.74	0.09	10.52	0.09	8.16	0.71	87.56
July.	0.74	0.11	15.4	0.08	10.53	-	-	-	-	0.63	85.36
Aug.	0.72	0.08	11.7	-	-	0.11	15.27	-	-	0.63	86.82
Sep.	0.79	0.11	14.5	0.15	18.42	0.10	12.18	-	-	0.64	80.76
Oct.	-	-	-	-	-	-	-	-	-	-	-
Nov.	-	-	-	-	-	-	-	-	-	-	-
Dec.	0.71	0.12	18.1	-	-	-	-	-	-	0.58	82.25

Table 3: *M. rosenbergii*-Types of Infections encountered and survival of post larval percentage in 2009

		Infection									
				Bacterial infection						% of	
	Stocking density	Protozoan infection		Filamentous Bacteria		Luminescence Bacteria		Fungal Disease		Survival	
Months	(Million)	Millions	%	Millions	%	Million	%	Million	%	Millions	%
Jan.	0.71	0.12	16.4	-	-	-	-	0.03	3.65	0.63	89.11
Feb.	0.67	0.15	21.7	0.07	10.15	-	-	0.06	8.72	0.59	88.90
Mar.	0.60	0.10	17.1	0.91	15.26	0.11	18.65	-	-	0.55	92.46
April.	0.64	0.12	18.6	0.08	12.19	0.19	21.74	-	-	0.55	86.38
May.	0.56	0.07	12.4	-	-	0.10	17.53	-	-	0.48	85.64
June.	0.71	0.15	20.7	0.12	16.74	0.08	10.54	0.04	5.46	0.63	89.36
July.	0.63	0.10	15.4	0.67	10.53	-	-	-	-	0.55	87.47
Aug.	0.53	0.09	13.7	-	-	0.10	15.27	-	-	0.56	88.28
Sep.	0.64	0.13	20.5	0.12	18.42	0.08	12.18	-	-	0.56	87.55
Oct.	-	-	-	-	-	-	-	-	-	-	-
Nov.	-	-	-	-	-	-	-	-	-	-	-
Dec.	0.58	0.16	18.1	-	-	-	-	-	-	0.51	87.82

the month of February and June. Lowest percentage of 11% observed in the month of August, 2010. Bacterial infection were noticed in the zoeal stage are shown in Table 2. A maximum of 18% infection due to filamentous bacteria was noticed in these larval stages. In case of luminescence bacteria, the maximum percentage of larvae affected were 19% in the month of May, 2010. The percentage of larvae infected with luminescence bacteria are shown in table. Fungal infection was also noticed in these stages in all the year of study and severe infection in this case was seen in this year when 18% of larval population were affected with this disease (Table 2)

Stage: Post Larvae: Incidence of infection due to protozoan ciliates both filamentous and luminescence bacterial and fungal disease were also recorded in the early juvenile stages. The contagious diseases used to spread fast in the aquatic media from larval stages to post larval stages. The various details about the percentage of juveniles prawn infected are shown in Table 3. It was noticed in the present observations that prolonged rearing of the larvae and continuous operations of the hatchery or laboratory without frequent time gaps between cycles lead to the development of infections. It was observed that the juvenile stages relatively resistant

to disease when compared to the zoeal stages. It was also been observed that the development of infective diseases especially the luminescence bacteria was more prevalent when the post larval stages were fed with formulated feeds, such as egg custard by using squid meal as one of the component (Table 3).

Various antibiotics and disinfections were used to control the infection caused by ciliated and fungal diseases. It was observed that the ciliates were susceptible to 1-10 ppm in various zoea stages and post larval stages. Similarly 2-4 ppm of cupric sulphate was found to be useful for successful control of the ciliates in commercial operations; through water exchange followed each treatment. The percentage of survival of the juveniles after treatments is shown in tables. In case of filamentous bacteria, the ideal control measures uses find out where to treat the larvae with Erythromycin phosphate and Cifroflaxine at a concentration of 1.0-3.0 ppm for different larval and post larval stages. Luminescent bacteria were found to be susceptible for Furazolidon at a concentration of 3.0-4.0 ppm for the larval and post larval stages. Various fungal diseases were controlled by treating the larvae and juvenile with Treflan 0.25-0.50 ppm, Prefuran (Argent Chemicals, USA made) at a concentration of 0.5-1.0 ppm.

DISCUSSION

Disease is a major threat for any biological system against which man's fight is un-ending and in aquatic system prevention in the best remedy. In a hatchery system is infected with a disease it is better to discontinue the operation till the pathogen is eradicated by disinfection and drying. Disease and parasites of scampi larvae in the hatchery constitute a potential constraint on successful production. A large amount of data has recently accumulated on a variety of pathogen affecting both natural and cultured prawn. These agents include viruses, which have been most extensively studied, rickettisia and rickettsia like organisms, bacteria, fungi, protozoan and helminthes.

In the present study, an attempt is made to study the prevalence and controlling measures of bacterial, fungal and protozoan diseases of larval and post larval stages of *M. rosenbergii* in commercial scampi hatcheries. The present investigation revealed the occurrence of filamentous bacterial diseases (*Lecothrix*) on larval stages of *M. rosenbergii*, found to occur on body surface and internal organs. 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 system, 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, ciliates multiplies rapidly attacking the appendages of the swimming larvae of *M. rosenbergii*. Present studies reveal that the peritrichous ciliates infected larvae and post larvae of *M. rosenbergii* exhibited a woozy appearance, frequent jumping and circular swimming movements. Earlier studies have been shown that epi-commensal organisms if present in large numbers on the body surface and appendages cause difficulties in locomotion, feeding, moulting resulting in mortalities. Disease problems associated with *M. rosenbergii* include protozoan infestation (especially *Zoothamnium, Vorticella* and *Epistylis* spp.) fungal pathogens (such as *Lagenidium callinectes, Sirolpidium* spp. and *Fusarium solani*) bacterial problems following injury to the exoskeleton and molt arrest due to poor environmental conditions or nutritional deficiencies also reported by Johnson, [12], Vijayan *et al.* [13] and Sriwongpuk *et al.* [14].

Infection of filamentous bacterium *Leucothrix* spp., on periopods and other appendages might lead to mortality of scampi larvae because of hypoxia; similar observations were noticed in the present findings, that mass mortalities also was noticed in infection due to filamentous bacteria. Infection of protozoan ciliated on the gills of the larvae usually dons 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 anastomosis on the gills and mouth parts, causing the mouthparts immobile, preventing the larvae to feed activity; death was due to starvation.

Prevention and control of luminous bacterial disease in scampi are generally achieved by chemical treatment. Chemo-therupeutants may be applied in the feed or in the water. Usually antibiotics are mixed in the feed while disinfectants (and sometimes antibiotics as well) are applied in the water. To prevent luminous diseases in hatchery operations the best way out was found to filter the water through a bio-filter system prior to chemical treatment with 32-33% active chlorine at 10-15g/ton or laboratory grade formalin at 10-25 ppm. During treatment aeration is necessary. Antibiotics such as Oxytetracycline are found useful in the control. According to Cook [15], Colorni [16], Peng *et al.* [17],

Arcier *et al.* [18] and Nash *et al.* [19] the presence of *Epistylis, Zoothamnium and Lagenidium* in the hatchery probably not represented responsible for causing mass larval mortalities. Sporadic infection due to fungus is not very common but this was also found in experimental tanks and mass culture of *M. rosenbergii* larvae and juvenile. Since fungal infections may be introduced at various phases of larval development. From the present study, it appears the zoea and advanced zoea may be exposed to 0.25 to 0.5 ppm Treflan without adverse effects, on survival, infection rates were found very much under control.

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