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ESTIMATION OF MICRO-FLORA ASSOCIATED WITH DIFFERENT STAGES OF *MACROBRACHIUM ROSENBERGII* (de Man)

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ABSTRACT

The bacterial flora occurring in muscle, haemolymph, hepatopancreas and gill of brood, juveniles, water, eggs, larvae and rearing water were estimated by selective plate count technique for *Entrobacteriaceae, Streptococaceae, and Vibrionaceae* members. The total viable bacterial count was estimated by total plate count technique on nutrient agar. The total viable counts of bacteria were lowest in water from ($6.10x10^2$ CFU/mL) and highest in egg ($6.06x10^8$ CFU/g). In brood the total counts were varying from $1.62x10^2$ CFU/g in muscle to $2.20x10^5$ CFU/g in gills. In juveniles, the total plate counts were varying from $2.8x10^4$ CFU/g in muscles to $3.67x10^8$ CFU/g in hepatopancreas. Selective plate counts show that *Enterobacteriaceae* members dominate in egg and gills of brood and hepatopancreas of juveniles. *Vibrios* were found to be dominant in water and larvae of rearing tank. Haemolymph of brood was sterile and did not contain any bacteria while muscle of juvenile was having very low count of total viable bacteria.

Keywords - *Macrobrachium rosenbergii*, egg, larvae, haemolymph, hepatopancreas.

INTRODUCTION

Freshwater prawn (*Macrobrachium rosenbergii*, de Man, 1879) is an important commercial species due to its good taste and is a valuable product for export. (Sharshar & Azab, 2008). Aquaculture of this species is fast expanding in India. With intensification, occurrences of diseases are also becoming more common. Outbreak of diseases in prawns is often associated with its water quality degradation and presence of pathogens in high number. Types of bacteria associated with farmed *Macrobrachium rosenbergii* and their levels are useful indicators of quality and safety of prawns (Lalitha and Surendran, 2004). A lower survival rate of *M. rosenbergii* during its larval stages often prevents the development of aquaculture of this species to its full economic potential (AQUACOP, 1977; Hanson & Goodwin., 1977). When usually measured physico-chemical parameters of water (NO_2-N, NO_3-N, pH, DO, temperature, salinity) are found to be maintained within acceptable range and the cause of mortality before metamorphosis can not be attributed to any apparent parasite, the involvement of pathogenic bacteria is often hypothesized, AQUACOP, 1977; Brock, 1983). Not enough

attention has been paid to analyze the bacterial flora associated with crustaceans like *M. rosenbergii* (Miyamoto *et al.*, 1983; Anderson, 1988). The present work was carried out to determine the number and types of specific kinds of bacteria associated with brood, juveniles, eggs, larvae and its rearing water along with their organs (gill, hepatopancreas, muscle and haemolymph) of *M. rosenbergii* in order to find out the kinds of microbes associated with its different stages of growth. This may help to take preventive measure against the harmful microflora.

MATERIAL AND METHODS

Sample collection: Brooders of *M. rosenbergii* were collected from a community pond of 6 ha area with a depth ranging from 1.2 m to 1.8 m located in suburban Mumbai, Maharashtra, India. They were transferred to freshwater prawn hatchery complex at CIFE, Mumbai, India with proper care to avoid any stress. The brooders for the study were selected randomly and were washed with 0.85% normal saline to remove the loosely bound micro-organisms. The eggs were then scooped out using sterile forceps into sterilized test tubes. Brooders were cut open aseptically to obtain a section of longitudinal muscles, hepatopancreas and gills using sterile scissors. The organs were weighed separately and homogenized aseptically with sterile saline. Rest of the brooders were left in spawning tanks containing brackish water with salinity range of 12-14 ppt. Eggs were hatched and early larvae were filtered and pooled in 1 litre sterilized beaker. One gram of larvae was taken as sample. At the same time, water from spawning tanks was also pooled and homogeneously mixed diluted serially to access the bacterial load in water. The remaining larvae in the tanks were reared for a period of 60 days and juveniles so obtained were also similarly processed as the brooders for muscles, hepatopancreas and gills. Haemolymph of juveniles and brooders were asceptically drawn with the help of disposable syringe and samples were processed immediately after preparation both from brood and juveniles.

Bacteriological analysis

All the glasswares used were sterilized in hot air oven at 180^{0}C for 60 minutes. Media, tips for micropipette, saline, water were sterilized at 121^{0}C under 15 lbs pressure for 15 minutes. Homogenates prepared in physiological saline were plated by spread plating technique on Nutrient Agar (Himedia, Mumbai) for total plate count. The incubation was done at 37^0 C for 24-48 hours and the counts were recorded to enumerate the Total Plate Counts (TPC). Homogenates were also plated on Kenner Faecal (KF) agar, Thiosulphate Citrate Bile Sucrose (TCBS) agar and Eosine Methylene Blue (EMB) agar for the counts of presumptive Streptococci, Vibrios and *Enterobacteriaceae* members. The enumerated values on these selective media were designated as numbers representing Streptococci , Vibrios and *Enterobacteriaceae* members.

Physico-chemical parameters of water

The pH, dissolved oxygen, temperature and salinity of the spawning tanks and larval rearing tanks were determined according to standards methods for the

examination of water and wastewater (APHA, 1998).

RESULTS

Physico-chemical characteristics of water

Physicochemical characteristics of spawning and larval rearing tanks were within the optimal range for *M. rosenbergii*. Temperature was 21±2^{0}C, pH values were ranging between 7.0-7.5, dissolved oxygen values ranged from 5.2-6.5 mgL^{-1} and salinity values were maintained between 12-14 ppt for larval rearing.

Bacterial counts

Total bacterial counts along with specific counts of *Enterobacteriaceae, Streptococcaceae* and *Vibrionaceae* in larval rearing water, eggs and larvae are presented in table-1, whereas the counts of the same in gills, haemolymph, hepatopancreas and muscles of juveniles and brood have been shown in table 2 and table 3.

In water tanks containing brood of *M. rosenbergii*, the total bacterial count observed was $6.10x10^2$ CFU/g. *Enterobacteriaceae* were highest in larvae $3.54x10^5$ CFU/g and lowest in egg $2.51x10^4$ CFU/g. *Vibrios* were recorded to be highest in water $3.90x10^4$ CFU/mL followed by in larvae $2.87x10^4$ CFU/g and then in egg ($1.75x10^2$CFU/g). In brood, highest bacterial load was observed in gills followed by hepatopancreas and muscles. The haemolymph of brood was found to be sterile and was devoid of any bacteria. The gills of the juveniles of *M. rosenbergii* were found to contain highest total count of bacteria as well as *Enterobacteriaceae* and *Vibrios*. While *Streptococcii* were not observed in gills, they were recorded highest in hepatopancreas. Vibrios were absent in muscles, haemolymph and hepatopancreas of juveniles.

Table 1. Bacterial counts in different organs of *Macrobrachium rosenbergii* juveniles

	Muscle	Haemolymph	Hepatopancreas	Gill
	CFU/g	CFU/g	CFU/g	CFU/g
Total count	$1.62x10^2$	NIL	$2.83x10^4$	$2.20x10^5$
Enterobacteriaceae	$1.42x10^2$	NIL	$6.1x10^5$	$1.02x10^5$
Vibrios	$3.0x10^1$	NIL	$1.0x10^2$	$4.2x10^2$
Streptococci	$8.4x10^1$	NIL	$1.13x10^3$	$1.83x10^3$

Table 2. Bacterial counts in different samples of *Macrobrachium rosenbergii* larvae, egg and water.

	Larvae	Egg	Water
	CFU/g	CFU/g	CFU/mL
Total count	$4.35x10^6$	$6.06x10^8$	$6.10x10^2$
Enterobacteriaceae	$3.54x10^5$	$4.85x10^4$	$2.51x10^4$
Vibrios	$2.87x10^4$	$1.75x10^2$	$3.90x10^4$
Streptococci	NIL	$1.23x10^3$	NIL

Table 3. Bacterial counts in different organs of *Macrobrachium rosenbergii* brood.

	Muscle	Haemolymph	Hepatopancreas	Gill
	CFU/g	CFU/g	CFU/g	CFU/g
Total count	$2.8x10^4$	$1.61x10^6$	$3.67x10^8$	$3.52x10^7$
Enterobacteriaceae	NIL	$1.28x10^6$	$8.35x10^6$	$1.34x10^7$
Vibrios	NIL	NIL	NIL	$6.36x10^3$
Streptococci	NIL	$1.38x10^4$	$5.53x10^6$	NIL

Discussion

Most of the diseases in *M. rosenbergii* are caused by opportunistic pathogens which are prevalent in rearing water environment (Lombardi and Labao 1991; Jayasree *et al.*,1999). There have been few studies of bacteria associated with the *M. rosenbergii* eggs, larval rearing medium and in different organs(Muscles, hepatopancreas, gills and haemolymph) of juveniles and brood. Highest counts of *Enterobacteriaceae* members were recorded in larvae followed by in eggs and in water. On the other hand, *Vibrio* counts were recorded to be highest in water followed by larvae and the least was recorded in eggs. Higher number of *Enterobacteriacea* in larvae may be because of rich organic matter content of water due to feed. Highest *vibrio* count was seen in larve, followed by water and least was seen in eggs. Moderately high *Vibrio* counts in water may be due to salinity content of brackish water (rearing medium) as most of the *Vibrio* require salt to grow. It is lowest in eggs because though the female become berried in freshwater where the salinity content is 0-0.5 ppt it requires brackish water to hatch. Therefore, addition of sea water to fresh water may favour the growth of *Vibrio* coupled by addition of feed (egg, mussel meat, artemia). The lower *Vibrio* count in water than the larvae indicates the succession of bacterial population. These bacteria have also been reported by Colorni (1985), and Lalitha and Surendran (2004) from *M. rosenbergii* larvae in rearing facility and culture pond respectively. In different organs of brood total counts were highest in gills followed by hepatopancreas and muscles. Since gills filter the water and remain in direct contact with the rearing medium, it has the possibility of harboring more bacteria than present in its surrounding. The specific counts of *Enterobacteriaceae* were highest in hepatopancreas, while *Vibrio* counts were lower than Streptococci. Uddin *et al.* (1998) have also reported occurrence of *Streptococci* and *Vibrio* in hepatopancreas and haemolymph of *M. rosenbergii*. The haemolymph of brood was sterile, that of the juveniles contained *Enterobacteriaceae* members and *Streptococci*. The sterile brood haemolymph indicates the healthy condition of brood while probably lower resistance of the juveniles to get rid of the bacteria. On the other hand, it may indicate that the brood had better efficiency to eliminate the bacterial population.

The kinds of bacterial population are indication of quality of food (Gennari *et al.*, 1998). The association of *Aeromonas, Vibrio* with the brood and juveniles may result in black spot necrosis in *M. rosenbergii* (Lambardi and Labao, 1991). Further, their presence along with *Vibrios* also indicates potential risk to human beings, if their pathogenic members are present. Relatively higher counts of bacteria from water to eggs and larvae may be because of increase in organic matter content due to feeding, while the decreased counts in brood than juvenile may be because of role of their immune system to remove these bacteria. Though, species level detection would reveal further information, it is important that the *Vibrios*, *Streptococci* and the members of *Enterobacteriaceae* are associated with different stages of growth which can pose potential risk to animal and human.

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