

PATHOBIOLOGICAL STUDIES ON CULTURED POPULATIONS OF THE FRESHWATER PRAWN, *Macrobrachium rosenbergii* (DE MAN, 1879), MARGARITA ISLAND, VENEZUELA

Estudios Patobiológicos en Poblaciones Cultivadas del Camarón de Agua Dulce *Macrobrachium rosenbergii* (De Man, 1879), Isla de Margarita, Venezuela

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ABSTRACT

Studies on the presence of diseases and parasites affecting larvae, post-larvae, juveniles and adults of *Macrobrachium rosenbergii*, cultured in Margarita Island, Venezuela, were undertaken. The presence of peritrichous colonial ciliates identified as *Epistylis* and *Vorticella* spp., and suctorians identified as *Acineta* and *Tokophrya* spp., occurring as ectocommensals or epibionts was confirmed. These organisms were encountered on the gills and exoskeleton of the four stages of growth examined. The presence of the cyanophyte algae *Anabaena* and *Lyngbya* spp., and the chlorophyte algae *Cosmarium* and *Ulothrix* spp., was also detected. Trichomes of filamentous bacteria identified as *Leucothrix* sp. were encountered in samples of the gills and exoskeleton of post-larvae and adults. Bacteriological studies on larvae from stages II and III, and of the water used for the larvae, revealed the presence of an abundant bacterial flora, in which representatives of the genera *Aeromonas*, *Arthrobacter*, *Flavobacterium*, *Moraxella*, *Pseudomonas* and *Vibrio* were present. A more detailed study of the aeromonads, pseudomonads and vibrios showed that the isolates belonged to the species *Aeromonas formicans*, *Pseudomonas alcaligenes* and *Vibrio anguillarum*, respectively. Fungi imperfecti, identified as *Fusarium* sp., were detected in specimens of post-larvae and adults, and

the organism was successfully isolated from the gills on Sabouraud dextrose agar plates.

Key words: *Macrobrachium rosenbergii*, pathobiology, infestation, Venezuela.

RESUMEN

Se realizaron estudios sobre ocurrencia de mortalidad y presencia de parásitos afectando larvas, postlarvas y adultos de *Macrobrachium rosenbergii*, cultivados en la Isla de Margarita, Venezuela. Se observó la presencia de ciliados peritricos coloniales identificados como *Epistylis* y *Vorticella* spp., así como se confirmó la ocurrencia de los ciliados suctorios *Acineta* y *Tokophrya* spp. como ectocomensales o epibiontes. Estos organismos fueron encontrados en las branquias y exoesqueleto de los cuatro estadios de crecimiento examinados. También se detectó la presencia tanto de algas cianofitas *Anabaena* y *Lyngbya* spp. como clorofitas *Cosmarium* y *Ulothrix*spp. Trichomas filamentosos pertenecientes a la bacteria *Leucothrix* sp. fueron encontrados en muestras de branquias y exoesqueleto de postlarvas y adultos. Los estudios bacteriológicos en larvas de los estadios II y III, así como del agua utilizada para su levantamiento, revelaron la presencia de abundante flora bacteriana, con predominancia de representantes de los géneros *Aeromonas*, *Arthrobacter*, *Flavobacterium*, *Moraxella*, *Pseudomonas* y *Vibrio*. Un estudio más detallado de aislamientos de aeromonas, pseudomonas y vibrios resultó en las especies *Aeromonas formicans*, *Pseudomonas alcaligenes* y *Vibrio an-*

guillarum, respectivamente. El hongo imperfecto *Fusarium* sp. fue detectado en especímenes de postlarvas y adultos, siendo exitosamente aislado de las branquias en placas de agar dextrosa Sabouraud.

Palabras clave: *Macrobrachium rosenbergii*, patobiología, infección, Venezuela.

INTRODUCTION

The palaeomonid genus *Macrobrachium* Bate, 1868 contains approximately 125 species, of which some are relatively small while others are capable of reaching more than 30 cm in length. These freshwater prawns are widely distributed in tropical and sub-tropical regions of the world, and several species of *Macrobrachium* are being cultured on a commercial basis [2, 31]. The aquaculture potential for the various species of *Macrobrachium* in Central and South America has been analyzed by New [29, 30]. This worker concluded that the greatest potential currently exists for *M. rosenbergii* (De Man, 1879), a South-East Asian species the production of which as an aquaculture activity is now generally available as a "technological package". Aguilera-Hernández et al. [1] have reported that *M. rosenbergii* is presently cultured in Argentina, Brasil, Colombia, Costa Rica, El Salvador, French Guiana, Guayana, Honduras, Mexico, Panamá, Surinam and Venezuela, in Latin America.

In common with other types of aquaculture operations, diseases and parasites are of importance in the culture of *Macrobrachium* spp. [5, 22]. The present research programme was undertaken with the aim of detecting the presence of ectocommensals or epibionts, internal parasites and fungi in populations of *M. rosenbergii* cultured in Margarita Island, Venezuela, and of studying the bacterial flora of the larval stages of development and of the water in which these were reared.

MATERIALS AND METHODS

Specimens of larvae, post-larvae, juveniles and adults of the species *Macrobrachium rosenbergii* were obtained from La Salle Foundation for Natural Sciences, Margarita Campus, Nueva Esparta State, Venezuela. Larvae and post-larvae were obtained from intensive hatchery culture with a 25% daily water exchange, while the juveniles and adults were cultured semi-intensively in concrete tanks with 8% daily water exchange. A total of 1,600 larvae, 1,600 post-larvae, 45 juveniles and 30 adults were examined to detect different types of epibionts, fungi, and parasites. Samples of Stage II and Stage III larvae, and samples of the water in which these had been reared, were taken to the Aquaculture Laboratory of the Instituto Oceanográfico de Venezuela, of the Universidad de Oriente.

All of the animals were subjected to a careful clinical examination [18]. For the detection of algae, and of bacterial and

protozoan epibionts, larvae were gently macerated between two glass slides for microscopical examination (400X) as wet mount scrapings from the exoskeleton and appendages. Wet mounts from the gills, hepatopancreas and abdominal musculature were similarly examined in the case of juvenile and adult prawns. The relative degree of infestation with epibionts was determined as recommended by Bell et al. [8] and Lightner [26].

Bacteriological examinations were undertaken on larvae produced in 70 liters capacity aquaria containing brackish water (salinity =16%), and on samples of this water. A salinity of 16% was used in all of the media utilized. The larvae were retained on an 80 µm filter, and were repeatedly washed with sterile artificial aged sea water. Weighed 0.5 g aliquots were prepared and aseptically homogenized in sterile brackish water, from which decimal dilutions were prepared for inoculation in 0.1 ml quantities onto plates of nutrient agar, incubated at 26°C for 48–96 hours. The water samples were used to prepare decimal dilutions and were treated in a similar manner. Bacterial colonies which subsequently developed were picked off and purified, and the individual isolates were identified on a basis of specific procedures [7, 12, 24, 25, 32, 36].

Attempts to isolate fungal pathogens detected were made using plates of Sabouraud dextrose agar (salinity = 1.6%), inoculated with pieces of gill tissue and with aliquots of the decimal dilutions prepared from the homogenized larvae.

The water quality was evaluated on a basis of temperature (°C), pH, salinity (%), nitrates (ppm N-N₂), nitrites (ppm N-NO₃) and ammonium (ppm N-NH₃), using samples obtained during the routine water monitoring of the experimental culture.

RESULTS

A high degree of infestation was determined on the gills and exoskeleton of the specimens examined. These infestations included the Cyanophyte genera *Anabaena* and *Lyngbya* causing grades 2, 3 and 4 infestations in post-larvae (25%), juveniles (30%) and adults (60%), and the Chlorophyte genera *Cosmarium* and *Ulothrix* causing grades 2 and 3 infestations in post-larvae (30%), juveniles (50%) and adults (25%), TABLE I.

Trichomes of filamentous bacteria identified as *Leucothrix* sp. were found causing grades 2 and 4 infestations in the gills and exoskeleton of adults and post-larvae, TABLE II.

Colonial peritrichous ciliates of the genera *Epistylis* and *Vorticella*, and suctorians of the genera *Acineta* and *Tokophrya*, were commonly detected as epibionts on the gills of post-larvae, juveniles and adults, with grades of infestation ranging from 3 to 4. The highest levels of infestation were detected in larvae (90%), and levels of infestation of 60% were found in post-larvae, juveniles and adults, TABLE II.

TABLE I
ALGAL EPIBIONTS DETECTED IN THE GILLS AND EXOSKELETON OF POST-LARVAE, JUVENILE AND ADULT
Macrobrachium rosenbergii IN MARGARITA ISLAND, VENEZUELA

Algae	Stage of Life Cycle Affected	Degree of Infestation	Localization	% Animals Affected
CYANOPHYTA	PL	2	Gills and exoskeleton	25
Order: Nostocales	J	3	Gills and exoskeleton	30
Genera: <i>Anabaena</i> and <i>Lyngbya</i>	A	4	Gills and exoskeleton	60
CHLOROPHYTA	PL	2	Gills and exoskeleton	30
Orders: Zygnematales and Ulotrichales			Gills and exoskeleton	50
Genera: <i>Cosmarium</i> and <i>Ulothrix</i>	A	3	Gills and exoskeleton	25

PL= post-larvae. J= juvenile. A= adults.

TABLE II
PROTOZOAN AND FILAMENTOUS BACTERIAL EPIBIONTS DETECTED IN THE GILLS AND EXOSKELETON OF LARVAL,
POST-LARVAE, JUVENILE AND ADULT *Macrobrachium rosenbergii* IN MARGARITA ISLAND, VENEZUELA

Organism	Stage of Life Cycle Affected	Degree of Infestation	Localization on Host	% Animals Affected
Phylum: CILIOPHORA	L,PL,J,A	3	Gills and exoskeleton (including appendages)	90 (L)
Class: Oligohymenophorea				60 (PL)
Sub-class: Peritrichia				60 (J,A)
Order: Peritrichida				
Family: Vorticellidae				
Genus: <i>Vorticella</i>				
Family: Epistylidae	L,PL,J,A	3	Gills and exoskeleton (including appendages)	90 (L)
Genus: <i>Epistylis</i>				60 (PL)
				60 (J,A)
Class: Kinetofragminophora	L,PL,J,A	4	Gills and exoskeleton (including appendages)	90 (L)
Sub-class: Suctorina				60 (PL)
Order: Suctorida				60 (J,A)
Family: Acenetidae				
Genus: <i>Acineta</i>				
Genus: <i>Tokophrya</i>	L,PL,J,A	3-4	Gills and exoskeleton (including appendages)	90 (L)
				60 (PL)
				60 (J,A)
Filamentous bacteria	PL	4	Gills and exoskeleton.	50
	A	2	Gills	30

L= larvae. PL = post-larvae. J= juvenile. A=adults.

No endoparasitic protozoans or metazoans were encountered in any of the freshwater prawns examined during this study.

The results of the bacteriological studies on the larvae and the water are summarized in TABLE III. It is apparent that the bacteria flora was composed of the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Aeromonas*, *Flavobacterium*, *Moraxella*, *Micrococcus*, *Photobacterium*, *Pseudomonas*, *Vibrio* and enterobacteria. Isolates of *Alcaligenes*, *Arthrobacter*, *Micrococcus* and *Photobacterium* spp. were obtained from the water, but not from the larvae, whereas isolates of *Aeromo-*

nas and *Vibrio* spp. were obtained from the larvae but not from the water.

The isolates of aeromonads, pseudomonads and vibrios were identified as the species *Aeromonas formicans*, *Pseudomonas alcaligenes* and *Vibrio anguillarum* ("*V. parahaemolyticus*") *sensu* Evelyn [19], which same comprised 43.33% of all isolates from the *Macrobrachium rosenbergii* larvae examined, TABLE IV.

Antibiotic sensitivity tests, using an impregnated disc method [7], showed that 50% of these strains of *Aeromonas*, *Pseudomonas* and *Vibrio* spp. were sensitive to penicillin,

TABLE III
DISTRIBUTION (%) OF THE PRINCIPAL TYPES OF BACTERIA ISOLATED FROM *Macrobrachium rosenbergii* LARVAE AND FROM THE WATER IN WHICH THESE HAD BEEN REARED IN MARGARITA ISLAND, VENEZUELA

Bacterial Type	Larvae (%)	Water (%)
Acinetobacter	13.8	12.9
<i>Aeromonas</i>	20.6	
<i>Alcaligenes</i>		3.2
Arthrobacter		6.5
Enterobacteria	3.5	9.7
Flavobacterium	27.6	29.0
Micrococcus		6.5
<i>Moraxella</i>	17.2	9.7
<i>Photobacterium</i>		32
<i>Pseudomonas</i>	10.3	19.3
Vibrio	3.5	
Unidentified isolates	3.5	

85.71% to chloramphenicol, and 78.57% to neomycin, novodoxylin and tetracycline, respectively.

Cases of mycotic invasion of the gills and appendages of *M. rosenbergii* 50% and of the exoskeleton of post-larvae 50% were detected. In all of these cases, the affected animals showed the presence of grayish-white to yellowish-colored lesions. Cane-shaped macroconidia were detected in wet preparations from the gills, and a tentative diagnosis of *Fusarium* sp. was made. The fungus was successfully isolated from the gills and surface lesions on plates of Sabouraud dextrose agar on which it produced an abundant cotton-wool type of mycelium, with yellowish or purplish-colored areas in the mycelium; conidiospores with oblong or ovoid septate macroconidia were produced, and served to confirm the diagnosis as *Fusarium* sp.

The basic water quality parameters of the larval rearing tanks are given in TABLE V. It can be appreciated that there was an increase in certain of the chemical characteristics (nitrites, nitates and ammonia), but no notable changes in the physico-chemical characteristics, when compared to the optimum values recommended for *Macrobrachium rosenbergii* culture [28].

TABLE V
PRINCIPAL CHARACTERISTICS OF POTENTIALLY PATHOGENIC BACTERIA ISOLATED FROM *Macrobrachium rosenbergii* IN MARGARITA ISLAND, VENEZUELA

Character.	Strain Nº													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gram.						-		-						
Morphology	Rod	Rod	Rod	Rod	Rod	Rod		Rod						
Flagella	P	P	P	P	P	P	P	P	NP	P	P	P	P	P
Nº flagella	2-3	1	1	1	1	-			NP	3	1-2	2	2-3	2
Growth at 5°C														
37°C	+									+	+	+	+	+
42°C												+		
Growth at 0% NaCl	+				+	+						+	+	
6% NaCl	+			+	+	+					+	+	+	
8% NaCl		+			+	+					+	+	+	
10% NaCl		+			-	-	-		+	+	+/-	+	+	+
Glucose O/F	NR	F	F	F	F	F	F	F	AL	AL	AL	AL	AL	AL
Gas from glucose	-				-	-	-	-						
Acid from glucose	-	+			+	-	-	-				-		
Add from saccharose		+	+		+									
Acid from lactose	-	+		-	+	-	-	-					-	
Acid from mannose	+			-	+	-	-	-						
Acid from arabinose				-	-	-	-	-	+	+++	-	+/-	+/-	+/-

JABLE IV (Cont.)

Caracter.	Strain N°													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Arginine dehidrolase									+			+		
Lysine descarboxylase							+	-	-	-	-	-	-	-
Omithine descarboxylase							+	+/-	+	+/-	+	+	+/-	+/-
Gelatine liquefaction														
Lipasa production	+													
Starch hydrolysis	+/-	+	+	+										
Chitin hydrolysis	ND	+	ND				+	-	+	-	-			+/-
Oxidase	+	+	+	+			+	+	+	+	+			
Indole production	-	+	+	+	+	+	+	+	-	-	-			
Catalase		+	+	+	+	+	+	+	+	+	+	+	+	
Methyl red		+	+	+	+	+								
Nitrate reduction		+	+	+	+	+	+	+	-	+	+	+	+	
Nitrate utilisation	-	+	+	+	+	+	+	+	-	+	+	+	+	
Citrate utilisation	+	+			+	+	+	+	+	-	-	+	-	
H ₂ S production		+						+/-	-	-	-	-	-	
O/129 sensitivity	-	-	-	+/-	-	-	+	-	-	-	-	-	-	-

+: positive. -: negative. +/-: weak positive. NR: not reactive. F: fermentation. AL: alkaline reaction. ND: not determined.

TABLE V
MEAN VALUES OF THE PHYSICO-CHEMICAL WATER QUALITY PARAMETERS IN THE CULTURE OF *Macrobrachium rosenbergii* IN MARGARITA ISLAND, VENEZUELA

Stage of Life Cycle	Temperature (°C)	pH	Salinity	N-NO ₂ (ppm)	N-NO ₃ (ppm)	N-NH ₃ (ppm)
Larvae	25.2	8.3	12	0.6069	0.7168	0.9532
Post-larvae	25.2	8.5	0	0.5093	1.9001 *	1.9780
Juvenile	25.0	8.3	0	0.5133	2.4059	2.0455
Adults	25.7	8.0	0	0.6219	2.2334	2.3214

DISCUSSION

Diseases of aquatic organism, including decapod crustaceans, are not simple events but rather they occur as the result of subtle inter-actions between the animal, the aetiological agent and the environment. Wedemeyer [37] concluded that the environment is the least stable of these three factors, and unfavorable environmental changes can produce stress conditions which serve to make the animals more susceptible to infections or infestations by facultative pathogens. The occurrence of stress, therefore, is possibly the one single factor of greatest importance in relation to the occurrence of many types of infection and/or contagious diseases in prawns.

The findings of the present work indicate that the larval stages of *Macrobrachium rosenbergii* appear to be more susceptible to disease problems associated with mortalities than do the post-larvae, juveniles and adults. Similar observations were recorded by Goodwin & Hanson [20] in Hawaii (USA), who pointed out that the low survival rate of the larval stages frequently prevents exploitation of the full economic potential of the giant freshwater prawn. In the present programme, high larval mortalities occurred (~60%) possibly due, in part, to the presence of a heavy organic load in the water at the hatchery, which water appears to have contained numerous potentially pathogenic bacteria. Brock & Lightner [11] sustained that when the physico-chemical parameters of the water are within nor-

mal acceptable limits and no mortality due to parasites can be demonstrated, then facultatively pathogenic bacteria can often be shown to be the cause of such mortalities.

The bacteriological studies carried out on the larvae demonstrated the presence of three types of bacteria recognized as being potentially pathogenic to crustaceans and other aquatic animals, namely: *Aeromonas formicans*, *Pseudomonas aicaligenes* and *Vibrio anguillarum* (= "*V. parahaemolyticus*"). The occurrence of such microorganisms in relation to freshwater prawn larvae represents a potentially dangerous situation in the survival of the *Macrobrachium rosenbergii* [17]. *V. anguillarum*, in particular, is of special interest in view of the active chitinolytic properties detected in all of the isolates obtained during this work. Chitinoclastic or chitinolytic bacteria have been shown to be involved in a condition denominated "Shell Disease" in crustaceans [21, 22, 27]. In the same way, *A. formicans*, *Flavobacterium* sp., *Ps. piscicida* and *Vibrio* spp., have been reported in association with aggregation and mortalities of penaeid shrimp zoeae and mysids in culture conditions [4, 6, 33]. The fact that the bacterial isolates should have shown a high degree of susceptibility to various antimicrobials indicates that these bacteria had not been in previous contact with antimicrobials and, indeed, such substances were never used in the rearing facilities.

A frequently occurring condition detected here was an extensive secondary infestation of the gills by algae, epibiont protozoans and filamentous bacteria, the presence of which gave rise to lethargy and lack of normal reflex movements in the affected prawns. Similar observations have been made by Smith et al. [35] in *Macrobrachium rosenbergii* cultured in the USA. As Brock [9, 10] has previously indicated, the occurrence of low numbers of epibionts has a minimal effect on the host, since these organisms do not penetrate the cuticle or cause a localized inflammatory response in the invaded area. Trichomes of *Leucothrix* sp. were also found causing moderate infestations in the gills and exoskeleton of post-larvae and adults. Although the presence of numerous filamentous bacteria was demonstrated, without any pathological changes being detected in the tissues, the degree of infestation could increase in periods of stress and lead to serious effects in the tissues due to secondary invasion by facultatively pathogenic eubacteria. Heavier infestations, however, are of greater importance, particularly in the case of the gills, where the infestation can impede normal respiration and give rise to mortalities associated with asphyxia or hypoxia [16, 38]. Infestations associated with ciliates of the epistylid genera *Epistylis* and *Opercularia*, the vorticellid genera *Vorticella* and *Zoothamnium*, and suctorians of the acinetid genera *Acineta*, *Acinetides* and *Tokophrya*, have been reported from adult *Macrobrachium rosenbergii* cultured in ponds in Limón Province, on the Caribbean coast of Costa Rica [14, 15, 23].

The detection of infections in *Macrobrachium rosenbergii* in Venezuela caused by *Fusarium* sp. is of interest. These cases were detected chiefly in adult prawns, which had previ-

ously undergone damage to the exoskeleton, although no mortalities from this cause were observed. Burns et al. [13] isolated this genus of Fungi imperfecti from *M. rosenbergii* and concluded that the fungus is not readily transmitted from diseased animals to healthy ones in the same facility. Infections due to *Fusarium* spp. have also been reported from penaeid shrimps [3, 34, 39].

On a basis of the results obtained during the execution of this study, the following recommendations have been made with reference to the prevention of disease problems in *Macrobrachium rosenbergii* culture operations in Venezuela or other tropical regions: (1) Careful attention must be given to maintaining optimum water quality parameters, with daily changes of at least 50% of the available water volume in hatchery facilities; (2) Uneaten food and other organic debris (including moulted shells) must be regularly removed from the water; (3) The population density of the prawns must be carefully monitored, and the numbers of animals maintained at an optimum level.

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