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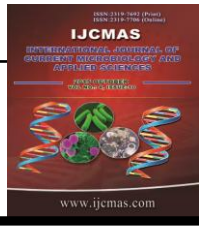

Original Research Article

Brown Spot Disease in *Penaeus kerathurus* Eggs and Larvae: A Case Report

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Penaeus kerathurus, Larvae, Eggs, Vibriosis

A B S T R A C T

Melicerthus kerathurus (Caramote prawn) is a very appreciated crustacean species, recently proposed as a species for restocking the natural population. The hatching and the early stages of larval development are the most delicate and generally more sensitive to the microbial attack. In this paper the authors reported an outbreak of brown spot disease among a wild broodstock of caramote prawn. Results showed that the etiological agent of disease was *Vibrio alginolyticus* that caused the disease in broodstock and in larvae and nauplii also, with 80 % of mortality rate. Accurate management protocols have been improved to achieve a good production results.

Introduction

Melicerthus kerathurus, commonly called Caramote prawn, is a very appreciated crustacean species and recently and been proposed as a species to breed at the end of restocking. The stages of hatching and the early stages of larval development, are the most delicate, during this period, generally the mortality is high and the species is more sensitive to the microbial attack (Prayitno & Lacthford, 1995). Vibrios, which represent the dominant part of the normal bacterial flora, during the stages of larval development of this species (Haamed, 1993), could be potential pathogenic (Lightner, 1993).

Vibriosis is the most widespread bacteria disease in shrimps which affects adults, larvae and post larvae, causing high mortality rate up to 80% in a few days. Generally vibriosis is attributed to the following species: *Vibrio alginolyticus*, *V. parahaemolyticus* and *Vibrio anguillarum*; these bacteria are opportunistic and are a part of the normal bacterial microflora of sea water and that associated with these organisms (Gomez-Gill et al., 1998; Mancuso 2014; Mancuso et al. 2013; Mancuso et al. 2010). They become pathogenic when there are environmental stressors (caused by pollution, manipulation of organisms, changes in temperature, salinity, etc.) and of course depend on the receptiveness of the host (Lightner & Redman, 1998).

Vibrios thanks to their chitinolytic activity, penetrate the shell, causing dark spots at the sites of infection, such as body surface area and the appendices (Mancuso 2014; Mancuso et al. 2013; Mancuso et al. 2010). *Vibrio alginolyticus* causes serious economic losses in farmed crustaceans and mollusks, affecting mainly shrimps, prawns and lobsters, causing infection to the carapax, pancreas and septicemia (Böhnel et al., 1999).

V. anguillarum, is one of the main etiological agent of vibriosis that can outbreak if the environmental factors (eg., temperature changes) or physiological (eg., stressors) change, transforming the bacteria into a highly pathogenic microorganism. Other diseases of crustaceans are related to larval luminescent bacteria such as *V. harveyi* (Diggles et al., 2000) and *Vibrio sp.*, often associated with a low larval survival and growth failure (Saulnier et al., 2000).

In this paper we reported the comments made on an episode of Brown Spot Disease recorded during an experiment of controlled reproduction of *M. kerathurus.*

The experiment was carried out inside the project MIPAF RIPATT "Experimentation of a strategy of repopulation with *Melicerthus kerathurus* in Coastal Zone between Capo San Marco and Capo Granitola" (MIPAF - CNR), in Sicily, Italy.

46 broodstock prawns were placed in the tanks of Aquaculture Experimental Plant of Institute for Marine Coastal Environment of Messina. After 7 days, the broodstock presented some melanosis and ulcers on the carapace, 2 days after there was the emission of the eggs and subsequently the hatching. Samples of haemolymph were drawn from adults using sterile siringes, swabs of lesions were withdrawn from adult specimens, samples of water were analyzed in order to identify the pathogen. Also, larvae were sampled and washed in a 20 ml volume of sterile (autoclaved at 121 C for 15 min) sea water three times for a duration of 1 min each, and macerated to a fine paste in a sterile glass following the Singh et al. 2006 protocol's.

All the samples were spread on TCBS agar (Oxoid) for the detection of Vibrios and on Marine Agar (Microbial Diagnostics) for counting cultivable heterotrophic bacteria. All strains were isolated in pure culture and were performed biochemical tests (oxidase, catalase, O129) and physiological (growth at different temperatures and salinity). The strains were identified by the use of the miniaturized system API 20E (bioMérieux), moreover the enzymatic activity was assayed using the API ZYM (bioMérieux) and finally, in the identified strains, was performed the antibiogram. The prawns emitted 158.000 eggs, the temperature was 27.8°C and salinity 37 ‰, the hatching rate was 17.4% and the percentage of larval mortality was 80%.

The necropsy showed that the broodstock presented melanosis of the carapax, sometimes accompanied by ulcers of greenish-brown on the post-rostral teeth, chest, abdomen and, in some specimens, pereiopods, with ulcers even deep greenish-brown.

Either the embryos inside the eggs and the nauplii (First Nauplius, N1) showed the ends of the appendages reddish (Figs.1,2). After hatching, were recorded setae and furcal spines missing and malformed and outbreaks on the body. The analysis carried out have led to the identification of *V. alginolyticus* strain both in lesions than in the water tank. The tests carried out with the API ZYM resulted in the presence of

chitinases (N-acetyl-β-glucosaminidase) typical of these bacteria, together with other enzymatic activities (Table 1). The results of sensitivity tests showed that the strains were susceptible to flumequine, sulfametazolo/ trimethoprim and enrofloxacin and were resistant to tetracycline, oxytetracycline and amoxicillin, in agreement with the results obtained from Hörmansdofer et al. (2000) and Singh et al. 2006.

Our study confirms that the presence of Vibrios is both in water tank and in the broodstock. The bacteria were found also in the healthy specimens, confirming the fact that these microrganisms are normally present in the bacterial marine flora and their opportunist nature also according to Ruangpang and Kitao (1991) and Vandenberghe et al. (1998). These opportunistic bacteria cause various kind of diseases if the organisms, especially during the early stages of development (eggs or larvae), are subjected to stress such as: unstable or sub-optimal environment, high stocking densities or an inadequate farming methods.

Previous studies carried out in Taiwan India, showed episodes of white spot disease in *Penaeus monodon* specimens farmed in extensive (Lee et al 1996, Selvin and Lipton 2003). Vibrio alginolyticus has been frequently isolated from diseased shrimp as the etiological agent of vibriosis and has been described as a principal pathogen of both penaeids and non - penaeids (Lightner 1988; Baticados, Cruz-Lacierda, de la Cruz, Duremdez-Fernandez, Gacutan, Lavilla-Pitogo & Lio-Po 1990; Mohney, Lightner & Bell 1994; Lee, Yu, Chen, Yang & Liu 1996; Mancuso et al. 2010; Mancuso et al. 2013; Mancuso 2014). A profound relationship between the abundance of members of the family Vibrionaceae and larval mortality (Singh 1990) and the predominance of Vibrio in eggs, larvae and post-larvae of *M. rosenbergii* (Hameed, Rahaman, Alagan & Yoganandhan 2003) was reported. Also *Vibrio harvey* could affect crustacean larvae (Diggles et al., 2000) and *Vibrio sp*. Are, often, associated with a low larval survival and growth failure (Saulnier et al 2000).

Karunasagar et al. 1994 investigated the mass mortality of *P. monodon* larvae reared in laboratory, and isolated a strain of *V. harveyi* resistant to the antibiotics. *V. harveyi* was, also, reported as etiological agent of Vibriosis in larvae of *P. indicus* (Prayitno and Lacthford 1995) and in larvae of *P. monodon* (Lavilla-Pitogo et al., 1990).

The operations of capture, transport and handling in general, must be carried out with extreme caution in order not to cause micro-cracks to the carapace, which can become infected for the presence of Vibrio species opportunist. These precautions are especially important in the case of individuals for breeding, since the early transmission of the disease (eggs and larvae) causes high mortality, as recorded in this case. In our case, the short time between capture and deposition suggests that the disease was provoked by the capture, handling and transport. In particular the transport, that was effectuated without any refrigeration, may have played the main role as stressful factor triggering the disease. And no doubt, that the shrimp may harbor in their normal microbial flora species of Vibrio (Gomez-Gil et al., 1998), without suffering in nature, but the captivity conditions could induce stress that cause the proliferation of pathogens and the consequently outbreak of diseases. It is therefore necessary to optimize the capture operations and the transport of subjects in order to minimize stress.

Table.1

Api zym	
Alcaline phosphatases	+++
Esterases (C4)	++
Esterases lipases (C8)	+++
Lipases (C14)	+
Leucine arilamidases	+++
Valine arilamidases	++
Cystine arilamidases	---
Trypsine	++
α-chimotrypsine	+++
Acid phosphatases	+++
Naftol-AS-BI-phosphohydrolases	++
α-galattosidases	---
β-galattosidases	---
β –glucuronidases	---
α-glucosidases	---
β-glucosidases	---
N-acetil-β-glucosaminidases	++
α –mannosidases	---
α –fucosidases	---

Fig.1 Adult of P. kerathurus diseased

Fig.2 Diseased embryo

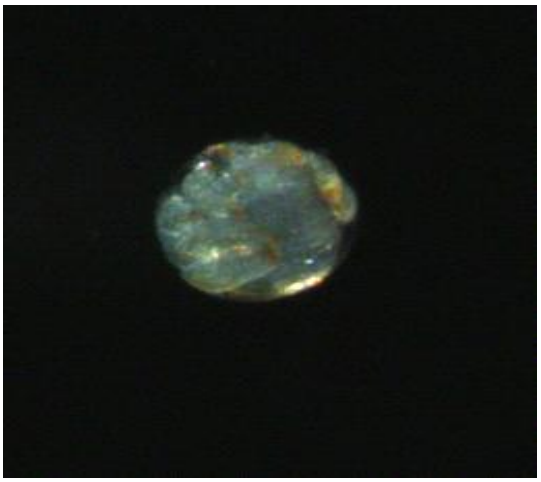

Fig.3 First Nauplius (N1)

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