J. Mar. Biol. Ass. India, **49** (2) : 257 - 259, July - December 2007

Note

Luminous vibriosis in a shrimp hatchery near Visakhapatnam, east coast of India

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Abstract

Luminous vibriosis and the sources/routes through which it entered the tiger shrimp (*Penaeus monodon)* hatchery near Visakhapatnam was studied during June 2006 – February 2007. Five larval cycles of *Penaeus monodon* were examined. *Vibrio harveyi* was present in all the luminous vibriosis samples. The natural seawater, the "carrier" brooder shrimps, the ineffective sterilization methods and unhygienic handling practices were found to be the major sources of luminous vibriosis in sterile shrimp hatchery facilities.

Microbial diseases cause serious financial losses to the shrimp farms. Viruses and bacteria are the key agents in causing mass mortalities in shrimp hatcheries and farms. Closure of shrimp hatcheries due to infections by luminous vibriosis is a recurring problem. Information is available on the occurrence of luminous vibriosis in shrimp culture systems of Andhra Pradesh (Nair *et al.,* 1979; Perumalsamy *et al.,* 1981, Ramaiah and Chandra Mohan, 1987, 1992; Ramesh *et al.,* 1987, Karunasagar *et al.,* 1994, 1996 Vinod *et al.,* 2006 and Kumar *et al.,* 2007). An attempt has been made to confirm the occurrence of luminous vibriosis in *Penaeus monodon* hatchery with two objectives: (i) identification of the luminous vibriosis species and (ii) identification of the possible sources/routes through which the luminous bacterium enters the sterile shrimp hatchery facilities.

Materials and methods

The present study was carried out in M/s. Siri Aqua Farms Pvt. Ltd. (Palmanpeta, Tuni, Andhra Pradesh) during June 2006 to February 2007. Five larval cycles of the tiger shrimp, *Penaeus monodon* were monitored (Cycle I: June, 2006; Cycle II: July, 2006; Cycle III: August 2006; Cycle IV: December 2006-January 2007 and Cycle V: January - February 2007). In each cycle, the samples for bacteriological studies were collected in duplicate. The samples selected for each cycle were natural seawater, treated seawater, brooder tank water, brooder surface swab sample, nauplius I stage, nauplius tank water, algal feed (*Chaetoceros*) sample, algal tank water, zoea I stage, zoea I stage tank water, mysis stage I, mysis stage I tank water, postlarva 1 stage, postlarva I stage tank water, *Artemia nauplii* feed sample, *Artemia nauplii* tank water, postlarva 8 stage and postlarva 8 stage tank water.

Water samples (100 ml) were collected aseptically into autoclaved bottles, which were again sterilized in an oven at 160° C. Live larval and feed organisms were collected at random and were homogenized aseptically using normal saline. The aseptically prepared samples were inoculated into *Vibrio* specific thiosulphate–citrate–bile salt–sucrose agar (TCBS medium, HiMedia) and were incubated at 33° C. The obtained cultures were enumerated for luminous bacteria in the dark chamber. The luminiscent colonies were aseptically grown in nutrient agar (HiMedia) and were identified using Bergy's manual (Holt *et al.,* 1994). Temperature was measured using 0.1° C sensitive hand-held thermometer. Salinity and dissolved oxygen were determined adopting Knudsen and Winkler methods (Strickland and Parsons, 1972). The pH was determined by a pH digital meter (Systronics).

Results and discussion

The luminous vibrio samples based on the morphological, ecological, biochemical and staining (Table 1) properties were identified as *Vibrio harveyi. Photobacterium phosphoreum, P. leiognathi, Vibrio fischeri and V. harveyi (*Family Vibrionaceae) are the common species that are responsible for marine bacterial bioluminisence. The ecological distribution of these four species in marine waters further confirms that *V. harveyi* is the most dominant luminous vibrio in the coastal waters. Ramaiah and Chandramohan (1992) while discussing the ecology and biology of luminiscent bacteria, pointed out that *V. harveyi* occurs up to 500 m. Below 500 m depth, *V. fischeri* and *P. phosphoreum* are more common. *P. leiognathi* occurs in the light glands of leiognathid fishes. The work of Karunasagar *et al.* (1994, 1996), Vinod *et al.* (2006) and Kumar *et al.* (2007) substantiate the occurrence of *Vibrio harveyi* in tiger shrimp hatchery facilities.

Table 1. *Morphological, ecological, biochemical and staining properties of luminous vibrios examined*

Character/parameter	Result
Size	4-5µm
Shape	Curved rod
Motility	Motile
Swarming	Absent
Gram's staining	Gram negative
Temperature	30-33°C
Arabinose	-
D-Mannose	+
D-Mannitol	+
Nitrate reduction	+
Oxidase	+
Lipase	+
Lysine decarboxylase	+
Ornithine decarboxylase	+
Bioluminescence	+

The luminous vibrios were present at low densities (mean: 49 cfu.ml^{-1}) in the natural seawater throughout the study period (Table 2). They were absent in treated seawater. Of the five larval cycles examined, the first larval cycle (Cycle I) was closed due to the occurrence of luminous vibriosis in the nauplii and zoeal tanks. The remaining four cycles were completed successfully. In all these five cycles, the brooders harboured luminous vibrios (504 cfu. ml^{-1}). Consequently the brooder tank water was also contaminated with luminous vibrios (mean: 1908 cfu. ml^{-1}). The larval stages and their tank waters, the live feed and their tank waters were free from luminous vibrios in all the five cycles investigated except in Cycle V tank water with postlarva 8 (mean: 30 cfu.ml^{-1}). Occurrence of luminous vibrios in the tank water with postlarva 8 may be due to improper sterilization and disinfection of culture enclosures.

Studies conducted by Nair *et al.* (1979), Lapota *et al.* (1988), Ramaiah and Chandramohan (1987) and Ramesh *et al.* (1987) report the occurrence luminous vibrios in natural seawater. Hence natural water is a major source of luminous vibrios into the hatchery facilities. The absence of luminous vibrios in the treated seawater indicates that the measures taken to remove bacterial populations are effective. It is clear from this study that the brooder shrimps harbour luminous vibrios. Unhygienic practices in the brooder–handling operations or brooder tank water disposal and

Table 2. *Mean distribution (n=4) of physico-chemical parameters of culture water and luminous bacterial densities (cfu.ml^{-1}) in seawater during different larval cycles of* Penaeus monodon

Parameter	Cycle I	Cycle II	Cycle III	Cycle IV	Cycle V
Temperature (°C)	30.2	30.1	29.8	28.3	28.8
Salinity (‰)	29.8	31.0	31.1	27.9	29.1
pH	7.9	7.9	8.0	7.9	8.0
Dissolved oxygen (ml/l)	6.5	6.8	6.7	6.5	6.5
Natural seawater (cfu. ml^{-1})	36	51	70	23	64
Treated seawater (cfu. ml^{-1})	0	0	0	0	0

Table 3. *Mean density distribution (n=2) of luminous bacteria (cfu. ml^{-1}) in different stages of larval development of* Penaeus monodon

Larval Cycle	Brooder		Nauplius stage I		Zoea stage I		Mysis stage I		Postlarva 1		Postlarva 8		*Chaetoceros* feed		*Artemia* nauplii feed	
	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O
I	3500	325	1740	2	5	2	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
II	40	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
III	101	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IV	863	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V	988	75	0	0	0	0	0	0	0	0	30	0	0	0	0	0

W: culture water; O: culture organism, Ns: no sample

ineffective sterilization of brooder enclosures act as another major route for the entry of luminous vibrios into aseptic hatchery facilities. During Cycle I, luminous vibrios were recorded in nauplii and zoeal stages. This may be due to the unhygienic handling of contaminated organisms or due to ineffective sterilization of culture enclosures.

In conclusion it may be stated that the entry of luminous vibrios into sterile shrimp hatchery facilities may be from improper sterilization of natural seawater, unhygienic handling of "carrier" brooder shrimps and ineffective sterilization of culture enclosures.

Acknowledgements

The authors are thankful to the authorities of Andhra University, Visakhapatnam and M/s. Siri Aqua Farms Pvt. Ltd., Palanpeta, Tuni for the laboratory and culture facilities provided.

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Received: 16 January 2008
Accepted: 04 April 2008