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NOTE

White spot syndrome virus (WSSV) in *Litopenaeus vannamei* captured from the Gulf of California near an area of extensive aquaculture activity

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ABSTRACT: For the shrimp farming industry of Mexico, disease outbreaks caused by white spot syndrome virus (WSSV) are relatively recent. Efforts to control the virus are assisted by monitoring for its prevalence in aquaculture systems, but few attempts have been made to search for it in carriers from coastal waters. To search for WSSV carriers in the Gulf of California, we made surveys off the coast of Sinaloa, Mexico, in March 2001, November 2001, and September 2003 using polymerase chain reaction (PCR) assays and histopathology. WSSV-positive shrimp were detected only in November 2001, after hurricane Julliete. This suggested possible dispersal of WSSV to the marine environment from infected shrimp farms.

KEY WORDS: WSSV · *Litopenaeus vannamei* · Aquaculture · Gulf of California · Pacific white shrimp

INTRODUCTION

The shrimp-culture industry around the world has faced the problem of epizootics caused by more than 20 viruses (Lightner 1996). Of these, white spot syndrome virus (WSSV) has caused 100% mortality in many farms with consequent severe economic losses (Chou et al. 1998, Zarain-Herzberg 2000, De la Rosa-Velez & Bonami 2002). In Mexico, WSSV has been reported since 1999 in cultured *Litopenaeus vannamei* (also called *Penaeus vannamei*) and in the crab *Callinectes sapidus* from drain channels in shrimp farms (López-Félix 2002). It is well known that the proliferation of shrimp diseases is increased by growth of the farming industry. The effluent from 1 farm recycled from the surrounding marine environment by adjacent farms is a common mechanism of disease dispersion, especially via marine fauna, if there is not a strict control of water discharge from aquaculture facilities (Lightner 1996, Lyle-Fritch & Romero-Beltrán 2002). The objective of our research was to determine the prevalence of WSSV to captured *L. vannamei* from coastal waters adjacent to an area of high shrimp-aquaculture activity.

MATERIALS AND METHODS

Three samplings were carried out during the fishing season onboard commercial shrimp trawlers operating off the northern coast of the Mexican state of Sinaloa, where a large number of shrimp farms operate (Fig. 1). A total of 424 individuals were taken (Table 1). Using the catch sequence, shrimp were numbered consecutively by trawl and their pleopods were dissected and placed in vials containing absolute ethanol for subse-

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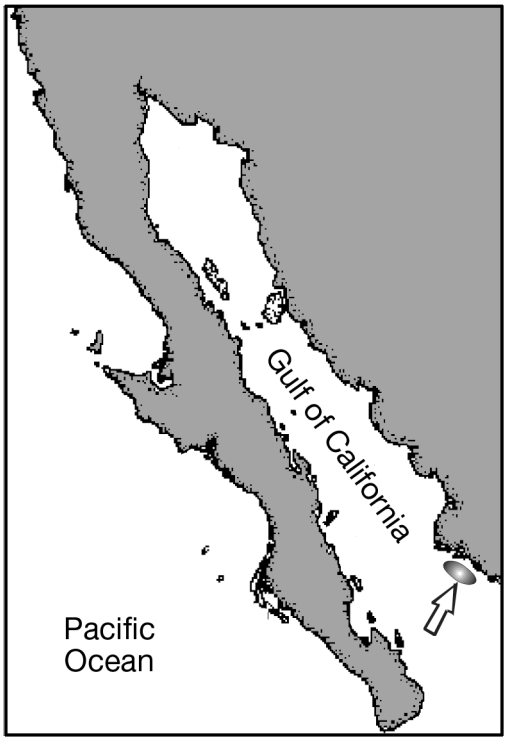

Fig. 1. Gulf of California showing sampling area (shaded ellipse) off the coast of northern Sinaloa, Mexico

quent polymerase chain reaction (PCR) analysis. For the histological analysis, the same shrimp were fixed with Davidson's solution while onboard the trawler and later transferred to 70% ethanol in the laboratory (Bell & Lightner 1988).

For histological analysis, gastric epithelia and gills were inspected for the pathognomonic eosinophilic to basophilic intranuclear inclusions characteristic of WSSV infections (Karunasagar et al. 1997, Kasornchandra et al. 1998). For PCR analyses, the IQ2000-WSSV kit was used following the manufacturer's instructions (Farming IntelliGene Technology). A first analysis was done in lots of 10 to 11 shrimp. When a positive result was obtained, individual shrimp were subjected to further PCR analysis. PCR products were observed in 2% agarose gels stained with ethidium bromide. In addition to the positive standard of the kit, we used a WSSV-positive shrimp from a nearby farm. All PCR runs also included a DNA-free sample for control.

RESULTS AND DISCUSSION

From the March 2001 sampling of 44 shrimp, no WSSV-positive samples were obtained using either PCR or histological analysis (Table 1). However, from the November 2001 sampling of 120 shrimp, 11 shrimp (9.2%) were WSSV PCR-positive and 1 of these specimens showed typical WSSV inclusions in the gastric epithelium and gills (Fig. 2). The shrimp with histological signs of WSSV gave a PCR result indicating a severe infection (Fig. 3) while 6 shrimp had light infections and 4 had very light infections according to the diagnostic kit's scale. All 11 of the PCR-positive shrimp came from the same trawl. Shrimp collected in 2003 (260) were negative for WSSV by both PCR and histological analysis.

In Mexico, WSSV was found in shrimp from Sinaloa farms in 1999 (López-Félix, 2002) and we found WSSV positive samples in the natural environment in 2001. The impact of other viruses such as infectious hypodermal hematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV) in both wild and cultured shrimp has been documented from the Gulf of California by Morales-Covarrubias et al. (1999) and Zarain-Herzberg & Ascencio-Valle (2001).

Although WSSV has been reported from Mexican shrimp farms, the scale of economic loss and the dispersion to wild populations has been poorly evaluated. Our sample of November 2001 with 9% WSSV-positive shrimp from the western coastal zone of the Gulf of California, where aquaculture activity is high, sug-

Table 1. Sampling locations, dates and number of wild *Litopenaeus vannamei* analyzed for presence of WSSV. (1 fathom = 1.83 m)

Location Date	Coordinates	Fathoms (no. of trawls)	No. shrimp	No. WSSV positive Histology	 PCR
Macapule March 2001	25° 23′ – 25° 25′ N, 108° 52′ – 108° 56′ W	5–10 (4)	44	0	0
Boca del Río November 2001	25° 15′ – 25° 20′ N, 108° 35′ – 108° 25′ W	5–10 (8)	120	1 (0.8%)	11 (9.2%)
La Palmita – Perihuete September 2003	25° 03′ – 25° 21′ N, 108° 23′ – 108° 60′ W	7–13.5 (11)	260	0	0

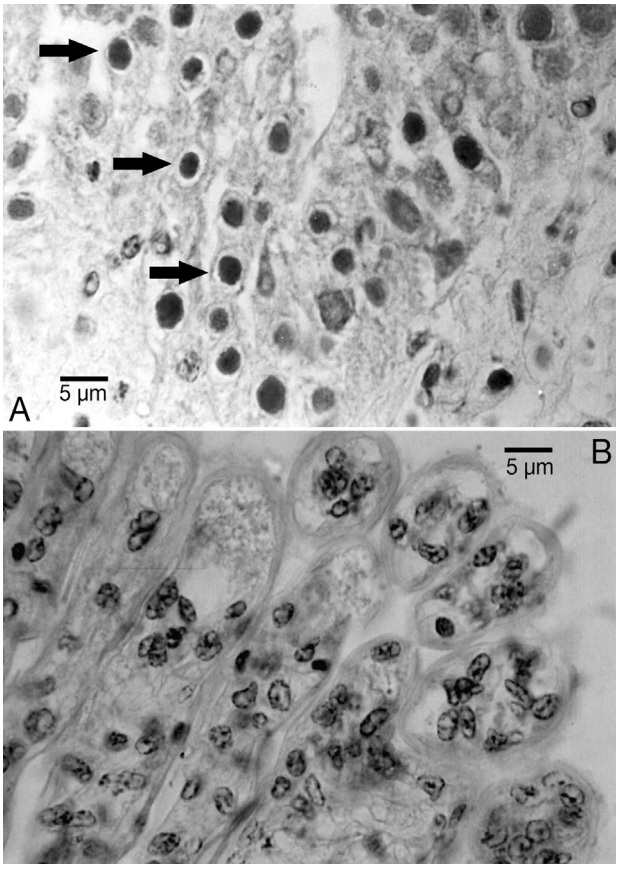

Fig. 2. *Litopenaeus vannamei.* (A) Photomicrograph of a gastric epithelium section of wild shrimp infected with white spot syndrome virus (WSSV) and showing typical WSSV inclusions (arrows). (B) Gill section of control (healthy) shrimp

gests that there may be a risk of farm contamination from the wild. We believe that WSSV-positive shrimp in the wild may originate from nearby shrimp farms as uncontrolled escapes. This contention is supported by the facts that hurricane Julliete struck the Mexican Pacific coast, including the Gulf of California, from 21 September to 30 October 2001 and resulted in severe damage from Mazatlan in Sinaloa province to Yavaros in Sonora province on 28 October. All shrimp farms in that region suffered severe damage, probably resulting in the dispersal of farmed shrimp, including those infected with WSSV, into coastal waters. If this contention is correct, the lack of WSSV-positive shrimp in the sampling from 2003 suggests that WSSV may have a low impact on wild white shrimp.

To minimize the spread of viral diseases in cultured shrimp, the Mexican government decreed an official edict NOM-EM-003-PESC-2000 prohibiting the importation of WSSV-infected shrimp. However, the presence of captured shrimp infected with WSSV is of concern. A more extensive study should be implemented to determine the prevalence of WSSV in wild carriers along the Gulf of California coast and the degree of risk it might present to shrimp farmers. Potential carriers include not only shrimp, but also other crustacean species such as *Callinectes* spp., *L. stylirostris* (also called *Penaeus stylirostris*), and *Farfantepenaeus californiensis* (also called *Penaeus californiensis*), which are also known to be susceptible to WSSV infection (Otta et al. 1999). To reduce transmission risk, continuous surveillance for WSSV in wild populations may be implemented and barriers constructed to prevent carri-

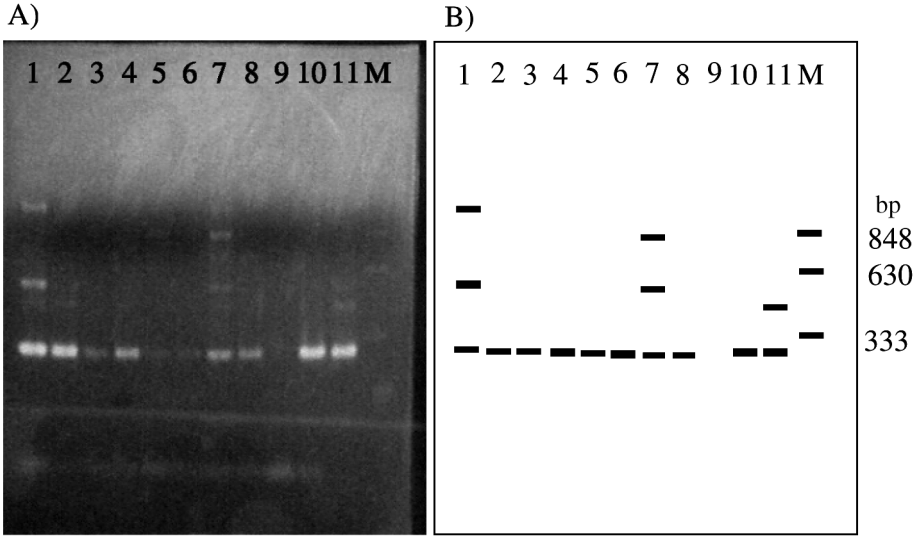

Fig. 3. *Litopenaeus vannamei.* (A) Scan and (B) drawing interpretation of agarose gel of PCR amplicons from 8 individual shrimp from a WSSV PCR-positive lot of 10 shrimp. Individuals showed severe (Lane 1), light (Lanes 2 to 6, 8), and very light (Lane 7) infections according to the kit manual. Lane 9: negative control. Lanes 10 and 11: positive standards (light and medium infections). M: molecular standard

ers from entering and leaving culture ponds. The results from our study may also give some insight into the relative potential impacts of shrimp diseases and overfishing on the current shrimp fishery industry crisis in the Gulf of California (Lyle-Fritch et al. 2001).

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