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Short communication

Prevalence of the infectious hypodermal and hematopoietic necrosis virus in shrimp (*Penaeus vannamei*) broodstock in northwestern Mexico

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ABSTRACT

The *Penaeus stylirostris* densovirus (*Pst*DNV or IHHNV) is the smallest of the known shrimp viruses. It causes severe mortalities in juveniles and sub-adults of the blue shrimp *Penaeus stylirostris*, while specimens of the white shrimp *Penaeus vannamei* infected by this virus exhibit reduced growth rates and negative effects on the feed-conversion rate (FCR). To date, no descriptive epidemiological surveys on the prevalence of this virus in shrimp broodstock have been performed. In this study, the prevalence of IHHNV in broodstock of the white shrimp *P. vannamei* from hatcheries on the northwest of Mexico region was estimated. Prevalence vary across different regions from high (63%) to low (6%) in shrimp broodstock. Several factors, as transport of pathogens by human activities, or the absence or implementation of ineffective biosecurity measures, may explain the observed differences. To the best of our knowledge, the present study is the first to examine the prevalence of IHHNV on broodstock.

1. Introduction

Global aquaculture production is growing rapidly, and the estimated shrimp production in the Americas accounts for nearly 20% of the global production, being Brazil, Ecuador, Honduras and Mexico the leading producers in the Western Hemisphere (Lightner, 2011). Although Mexico has an extensive coastline of 11,122 km, an estimated 90% of the shrimp farms are concentrated along the east coast of the Gulf of California (CONAPESCA, 2012).

The Mexican shrimp seed production relies on approximately 20 commercial hatcheries located in Northwestern Mexico, producing up to 10 billion post-larvae annually, which are mainly distributed in Sonora, Sinaloa, Chiapas, Tabasco and Tamaulipas (Fig. 1) (COSAES, 2013; CESASIN, 2012). During the early 1990s, the production of shrimp post-larvae in Latin America was based almost exclusively on the spawning of wild-caught broodstock, however, the occurrence of outbreaks caused by several viruses in shrimp-farming areas led many shrimp hatcheries to develop protocols for a sustainable supply of healthy

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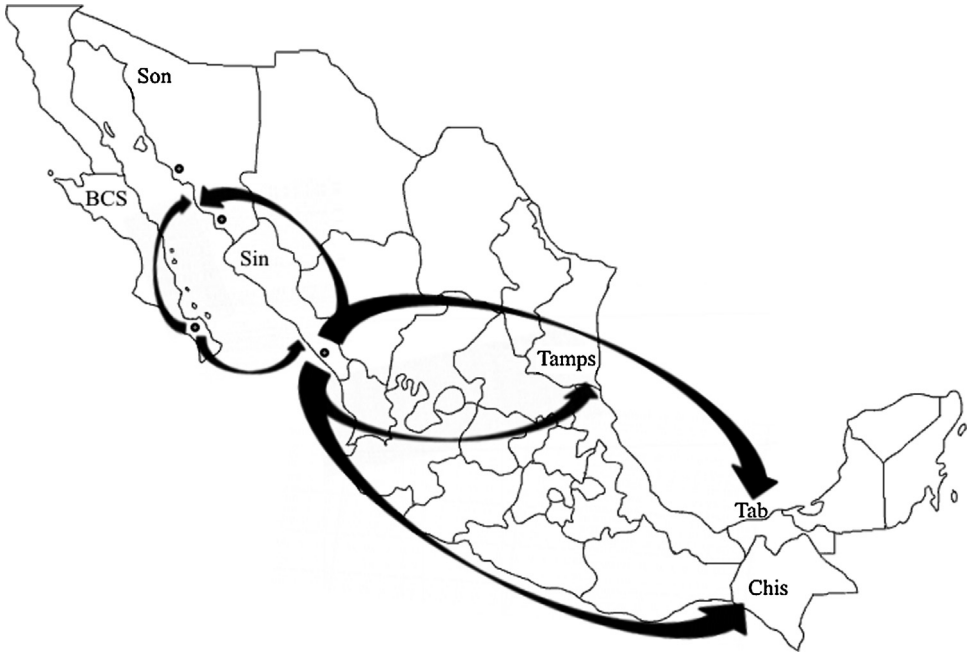

Fig. 1. Map of Mexico showing the locations of the five sampled hatcheries (filled circles). In Baja California Sur (BCS) samples were withdrawn from a single hatchery, the samples from Sonora (Son) and Sinaloa (Sin) were obtained from four different hatcheries. The arrows indicate the trade route of shrimp post-larvae.

domesticated stocks; thus, effective biosecurity and health management are considered the most critical of all biological requirements for successful broodstock rearing (Preston et al., 2007).

The *Penaeus stylirostris* densovirus (*Pst*DNV), a non-enveloped icosahedral virus with an average diameter of 22 nm (Bonami et al., 1990), is the smallest of the known penaeid shrimp viruses (Lightner, 2011). *Pst*DNV is also known as infectious hypodermal and hematopoietic necrosis virus (hereinafter referred to as IHHNV) and member of the family *Parvoviridae* (Mathews, 1982). Several reports have shown that shrimp populations may experience either horizontal or vertical transmission of IHHNV (Tang and Lightner, 2006; Vega-Heredia et al., 2011). Furthermore, IHHNV-infected shrimp *Penaeus vannamei* exhibit cuticular malformations of the rostrum and reduced growth rates associated to a chronic disease named "Runt Deformity Syndrome" (RDS), which may be associated to an abnormal glycolysis disorder known as Warburg effect (Galván-Alvarez et al., 2012). In addition, studies in *P. vannamei* show the negative effect on survival and the feed-conversion rate (FCR) of the IHHNV (Singhapan et al., 2004).

At present, since no effective treatments against penaeid shrimp viruses are available (Sánchez-Paz et al., 2012), strict and continuous epidemiological surveillance in broodstock has proven to be an invaluable barrier to avoid the dispersion of the Infectious Hypodermal and Hematopoietic Necrosis disease to shrimp farms. Few epidemiological studies have been conducted on farmed aquatic species (Corsin et al., 2002) and studies to determine the frequency of IHHNV in shrimp farms are scarcer. The aim of this study was to estimate the prevalence of IHHNV in shrimp *P. vannamei* broodstock, in northwestern Mexico.

2. Materials and methods

2.1. Sample collection and DNA isolation

To estimate the presence of IHHNV in shrimp broodstock, 150 organisms were randomly collected on each of 5 different commercial hatcheries identified by letters A through E (Fig. 1), according to the sample size formula of Cannon and Roe (1982) at a 95% confidence interval. This number corresponds to a population of 10,000 individuals per hatchery for an expected prevalence of 2%. A total of 750 individuals of shrimp broodstock (*P. vannamei*) were collected.

Hemolymph samples (100 μL) of each organism were withdrawn aseptically from the ventral sinus of each shrimp using a 1 mL syringe (27 gauge) containing 500 μL of anticoagulant solution (450 mM NaCl, 10 mM KCl, 20 mM EDTA-Na2, 10 mM HEPES pH 7.3, 850 mOsmkg^{-1}) (Vargas-Albores et al., 1993). Hemolymph samples of each laboratory were mixed in a pool from 3 specimens ($n = 250$ pools) and fixed in 500 μL of 70% ethanol. Surveys were conducted during the winter of 2011–2012 in hatcheries located in Baja California Sur, Sonora and Sinaloa. Samples were randomly taken from apparently healthy organisms, before the animals were transferred to the maturation units.

Shrimp genomic DNA was isolated by using the silica extraction kit provided with the commercial kit IQ Real Detection and Prevention System (GeneReach