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Detection of Necrotizing Hepatopancreatitis (NHP) in Wild Shrimp from Laguna Madre, Mexico by a Multiplex Polymerase Chain Reaction

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Short Communication

Detection of Necrotizing Hepatopancreatitis (NHP) in Wild Shrimp from Laguna Madre, Mexico by a Multiplex Polymerase Chain Reaction

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Abstract

The Laguna Madre in Mexico is a nursery, growth, and refuge area for shrimp and other wildlife species. These organisms support an important fishery for local industry (third place in Mexico), which may suffer diseases that are few studied. Wild shrimp were randomly collected from two sampling stations (Carrizal and Carbonera) once a month for one year (September 2007 to September 2008) to screen for virus and bacteria. The shrimp were taxonomically identified, organized in pools of three organisms per species and analyzed for the presence of WSSV, IHHNV, NHP and HPV by a commercial multiplex PCR kit (DiagXotics Inc. USA). All the wild shrimp species (*Litopenaeus setiferus, Farfantepenaeus aztecus*, and *F. duorarum*) were negative for the presence of virus. However, different pools of *F. duorarum* (prevalence of 15 and 5.6%) and *F. aztecus* (prevalence of 17 and 5%) were positive for NHP in both stations (Carrizal and Carbonera). This work is the first confirmation report of the presence of this bacterium on wild shrimp, which poses a potential threat to the local fisheries and aquaculture activities in the Laguna Madre and Gulf of Mexico.

Keywords: NHP, PCR, Wild shrimp

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บทคัดย่อ

การตรวจโรคการอักเสบของตับและตับอ่อนแบบเนื้อตายของกุ้งธรรมชาติ รอบบริเวณทะเลสาบ Madre อ่าวเม็กซิโก โดยวิธีมัลติเพล็กซ์ลูกโซ่โพลิเมอร์เรส

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ทะเลสาบ Madre ในประเทศเม็กซิโกเป็นแหล่งเพาะพันธุ์กุ้งและสัตว์ป่าธรรมชาติหลายชนิดโดยมีความสำคัญต่อกิจกรรมการประมงพื้นบ้าน 20 แห่ง (ลำดับที่ 3 ของประเทศ) ซึ่งอาจได้รับผลกระทบจากโรคต่างๆ ที่ยังมีการศึกษาไม่มากนัก การตรวจเชื้อไวรัสและแบคทีเรียในกุ้งธรรมชาติในครั้งนี้ทำโดยการรวบรวมกุ้งจากสถานีเก็บตัวอย่าง Carrizal และสถานี Carbonera เดือนละ 1 ครั้ง ตั้งแต่เดือนกันยายน 2007 ถึงเดือนกันยายน 2008 เป็นเวลา 1 ปี นำกุ้งที่รวบรวมได้มาจำแนกสายพันธุ์ ทางอนุกรมวิธาน แล้วทำการตรวจเชื้อ WSSV, IHHNV, NHP and HPV ในกุ้งแต่ละสายพันธุ์ สายพันธุ์ละ 3 ตัว โดยใช้ชุดทดสอบมัลติเพล็กพีซีอาร์สำเร็จรูป (DiagXotics Inc. USA) ผลการตรวจกุ้งธรรมชาติที่รวบรวมได้ทั้งสิ้น 3 สายพันธุ์ ได้แก่ *Litopenaeus setiferus*, *Farfantepenaeus aztecus* และ *F. duorarum* ให้ผลลบต่อการตรวจไวรัสในทุกตัวอย่าง แต่ให้ผลบวกต่อ NHP ในกุ้ง *F. duorarum* และ *F. aztecus* โดยพบในตัวอย่างที่ได้จากทั้งสองสถานี ทั้งนี้ รายงานนี้เป็นรายงานครั้งแรกที่มีการตรวจพบ NHP ในกุ้งธรรมชาติ 30 ตัว ในบริเวณในทะเลสาบ Madre ในอ่าวเม็กซิโกซึ่งอาจส่งผลกระทบต่อการประมงพื้นบ้านและกิจกรรมการเพาะเลี้ยงสัตว์น้ำในบริเวณดังกล่าว

คำสำคัญ: NHP PCR กุ้งธรรมชาติ

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Introduction

The world fishery production has slightly decreased while human consumption has increased (Bartley et al., 2006). This reduction in capture fisheries has been partly compensated by the fast growth of the aquaculture industry, where the commercial culture of penaeid shrimp is an important sector (Aguirre-Guzman et al., 2009). Shrimp culture conditions facilitate pathogen presence, growth, and their dissemination to the environment, neighboring farms-regions, and are responsible for the collapse of the shrimp industry in several areas of the world (Flegel, 2006; Aguirre-Guzman et al., 2009; Cock et al., 2009).

The worldwide transport of farmed shrimp to new culture areas has increased, where some organisms as *Litopenaeus vannamei*, *L. stylirostris*, *Penaeus monodon* are exotic species that may carry unusual pathogens, presenting a risk to the local fauna (Bartley et al., 2006; Río-Rodríguez et al., 2006; Freitas et al., 2007). Furthermore, shrimp processing plants, their byproducts and waste water help in the spread of pathogens (Gill, 2000) and emerging infectious diseases (Bartley et al., 2006; Wang et al., 2006). However, knowledge of the effects of established or foreign organisms and their pathogens on wild shrimp populations, and their impact on local fisheries is limited (Río-Rodríguez et al., 2006).

The Laguna Madre is an extensive lagoon system in the western coast of the Gulf of Mexico, which is a refuge, nursery and growth area for fish, birds, sea turtles, shrimp and other organisms with commercial significance. It is 200 km long (200,000 ha of shallow water) and supports an important fishery of *L. setiferus, Farfantepenaeus duorarum*, and *F. aztecus*. This last species is the main shrimp collected by artisanal and commercial fisheries along to the Gulf of Mexico, as well as the main species used in cage culture research in the Laguna Madre (Britton and Morton, 1989; Fernandez-Martinez et al., 2004; Río-Rodríguez et al., 2006).

Different pathogens have been detected in all developmental stages of cultured and wild shrimp, from post-larvae to adults (Flegel, 2006). However, in the Gulf of Mexico the agent responsible for necrotizing hepatopancreatitis (NHP) has only been detected on cultured *L. vannamei*, though its presence has been suggested on wild organisms (Río-Rodríguez et al., 2006). The signs of disease include mortality, lethargy, poor feeding and slow growth, anorexia, empty intestinal tract, softened exoskeleton and flaccid bodies (Lightner, 1996). The purpose of this study was to screen wild shrimp from Laguna Madre for the presence of WSSV, IHHNV, NHP, and HPV using PCR as a detection protocol.

Materials and Methods

Sample area and collection: Shrimp were collected from two stations [Carbonera (24°37´N-98°45´W) and Carrizal (24°55´N-98°27´W)] in the Laguna Madre (Figure 1), where this crustacean fishery is trapped in a castnet-like artisanal gear (charangas) (Figure 2). Two samples of twenty juvenile shrimp per station were randomly collected at night once a month from September 2007 to September 2008, [20 shrimp x 2 samples = 40 shrimp per station x 2 station = 80 shrimp per night x 12 month = 960 total shrimp per year]. There was no sampling in June due to the closed season in the shrimp fisheries. All sampling wild shrimp were stored in sterile containers with 70% ethanol (Vincent and Lotz, 2007) and transported in a polystyrene box to our Molecular Biology Laboratory (FMVZ, UAT). Shrimp were taxonomically identified and observed for signs of disease (Lightner, 1996).

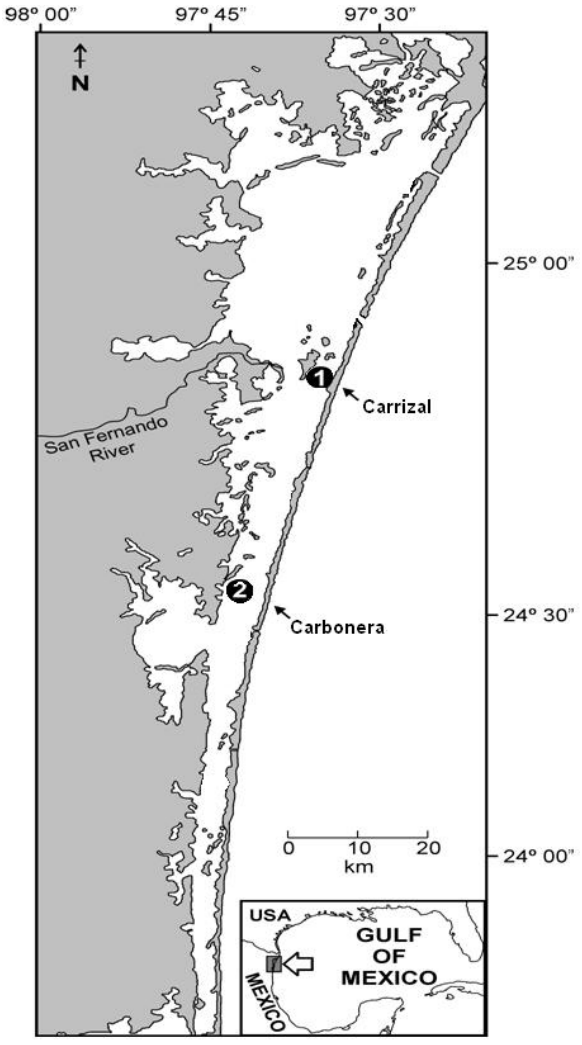

Figure 1 Location of sampling sites along the Laguna Madre of Tamaulipas (Mexico).

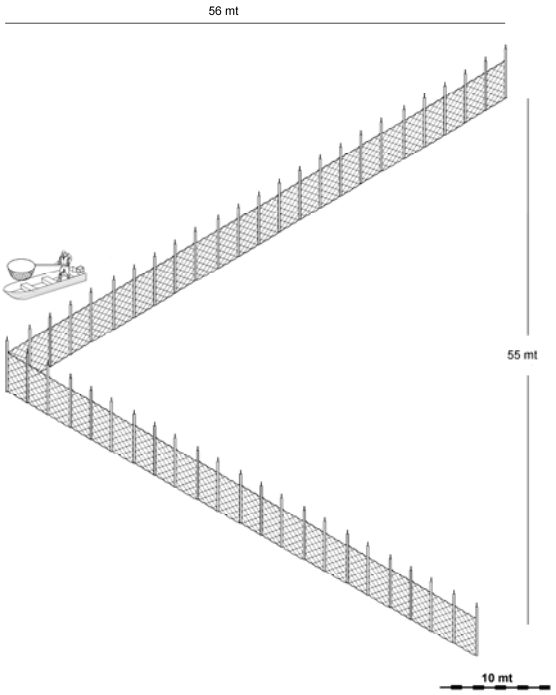

Figure 2 Charanga, an artisanal gear used in Laguna Madre, Tamaulipas for the shrimp fishery.

Polymerase Chain Reaction (PCR) Detection: The identified shrimp, from each month and sampling station, were pooled in groups of three organisms per species and independently analyzed for PCR. Each shrimp in a pool was dissected and its pleopods and hepatopancreas sampled, mixed and processed for DNA extraction using a commercial DNA isolation kit (Wizard SV Genomic DNA Purification System, Promega, USA). The extracted DNA was screened to detect the presence of microorganisms using the multiplex primer kit for WSSV, IHHNV, NHP, and HPV (DiagXotics, USA)(Ibarra-Gámez et al., 2007). The PCR was performed in a total volume of 25 µl (12.5 µl of Go Taq 2x, 6.5 µl of water), 5 µl of DNA, and 1 µl of multiplex primer mix for WSSV-IHHNV-NHP-HPV. The cycling protocol consisted of an initial 2 min denaturation at 95°C, followed by 40 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 55°C), and extension (90 sec at 72°C) with a final extension at 72°C for 7 min. The multiplex primer (DiagXotics, USA) kit produces amplicons of 401, 347, 312, and 255 bp (WSSV, IHHNV, NHP, and HPV respectively). It also has an internal shrimp DNA control of 506 bp, which is detected in the negative control (SPF shrimp DNA) and all shrimp tissue samples. Three microlitres of the amplified product were analyzed by electrophoresis (100V at 90-120 min) on a 1% agarose gel. Gels were visualized using a DIGI DOC-IT System. Recently, this PCR protocol was used, with positive results, in an intercalibration exercise of Mexican laboratories associated to shrimp diagnostics. This intercalibration was conducted by the Mexican government with the University of Arizona (Aquaculture Pathology Laboratory, USA).

Results and Discussion

The shrimp samples were taxonomically identified as *F. aztecus, F. duorarum* and *L. setiferus* (474, 318, 24 org. respectively; 816 total shrimp, 144 unidentified), all are species of commercial importance (Britton and Morton, 1989), and which showed no signs of disease. The results show that all wild shrimp pools were negative for WSSV, IHHNV and HPV. The prevalence of NHP in *F. duorarum* pools were 15 and 5.6% and *F. aztecus* were 17 and 5% from Carrizal and Carbonera, respectively. Figure 3 shows an agarose gel with the characteristic positive NHP amplicon (312 bp) detected by multiplex PCR. The 506 bp internal control can also be observed in the negative control (lane 3) and in wild shrimp samples (lanes 5, 6, 7). PCR positive status was reconfirmed with IQ2000 kit (Briggs et al., 2004) for NHP according manufacture instruction (data not show).

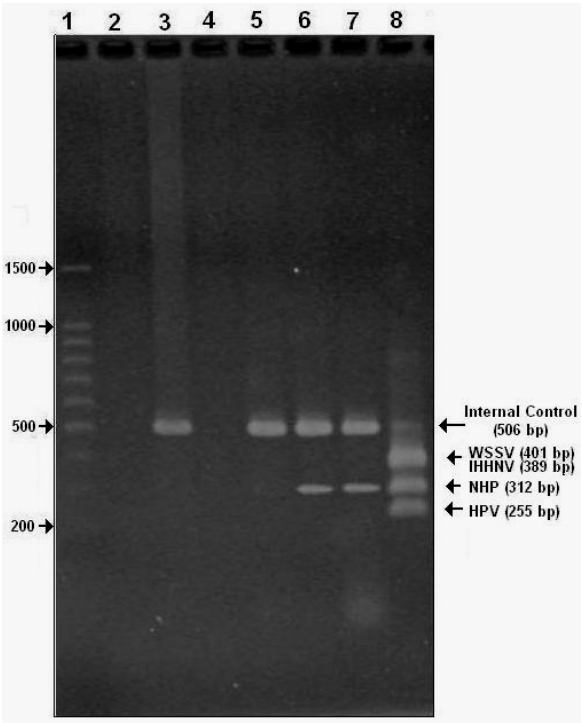

Figure 3 Agarose (1%) gel electrophoresis showing positive amplicons for NHP from wild shrimp sampled from the Laguna Madre, Tamaulipas using a Multiplex Primer kit (DiagXotics Inc. USA). 1: 100 bp molecular weight marker, 2 & 4: Sterile distilled water, 3: SPF shrimp DNA negative control template), 5: *Litopenaeus setiferus* sample , 6: *Farfantepenaeus aztecus* sample, 7: *F. duorarum* sample and 8: positive WSSV-IHHNV-NHP-HPV control template.

NHP has been a documented disease for America since 1985, affecting cultured shrimp such as *L. vannamei* and *L. stylirostris* (Vincent and Lotz, 2007). However, *F. aztecus, L. setiferus,* and *F. californiensis* (Holmes) are reported as hosts (Lightner, 1996; Vincent and Lotz, 2007), while *P. monodon* and *Fenneropenaeus chinensis* are reported as experimentally susceptible to NHP infection (Pantoja and Lightner, 2003). NHP is a pleomorphic intracellular α Proteobacterium, which infects epithelial cells in the hepatopancreatic tubules. NHP detection relies on different techniques, with PCR being the confirmatory protocol (Lightner, 1996). NHP has been reported in shrimp farms from the Pacific Coast of Mexico (Ibarra-Gámez et al., 2007) and was recently detected on shrimp farms in Campeche, Mexico (Río-Rodríguez et al., 2006). This bacterium has also been suggested as a possible pathogen for wild shrimp; however it has not been previously detected in wild penaeids (Río-Rodríguez et al., 2006). To our understanding, this work is the first report about the presence of necrotizing hepatopancreatitis (NHP) on wild shrimp, which is an important finding for future studies and show a high relevance for fisheries industry and aquaculture in Golf of Mexico.

The detection of NHP, and others pathogens, opens an opportunity to increase research on the costal lagoon about this and other diseases on wild shrimp populations, their biogeographical distribution, presence and/or establishment on shrimp and other crustacean species, their effect on shrimp fisheries, aquaculture, etc. Also, open an opportunity to study the spread mechanism and pathogen source (aquaculture, by-product, etc).

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References

Aguirre-Guzmán, G., Sánchez-Martínez, J.G., Campa-Córdova, A.I., Luna-González, A. and Ascencio, F. 2009. Penaeid shrimp immune system: a minireview. Thai J. Vet. Med. 39: 205-215.

Bartley, D.M., Bondad-Reantaso, M.G. and Subasinghe, R.P. 2006. A risk analysis framework for aquatic animal health management in marine stock enhancement programmes. Fish. Res. 80: 28-36

Briggs, M., Funge-Smith, S., Subasinghe, R. and Phillips, M. 2004. Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. Series title: RAP Publication - 2004/10 88 p.

Britton, J.C. and Morton, B. 1989. Shore ecology of the Gulf of Mexico. University of Texas Press, Austin, USA.

Cock, J., Gitterle, T., Salazar, M. and Rye, M. 2009. Breeding for disease resistance of Penaeid shrimps. Aquaculture. 286: 1-11.

Fernandez-Martinez, R., Rangel-Davalos, C., Ramirez-Flores, M., Gonzalez-Cruz, A. and Conde-

Galaviz, E. 2004. Survival and growth of brown shrimp *Farfantepenaeus aztecus* in cage culture in Laguna Madre, Tamaulipas, México. IX Aquaculture Latin American Congress, Villahermosa Tabasco, Mexico, p. 159 (Spanish version)

Flegel, T.W. 2006. The special danger of viral pathogens in shrimp translocated for aquaculture. Sci. Asia. 32: 215-221.

Freitas, P.D., Calgaro, M.R. and Galetti, Jr.P.M. 2007. Genetic diversity within and between broodstocks of the white shrimp *Litopenaeus vannamei* (Boone, 1931) (Decapoda, Penaeidae) and its implication for the gene pool conservation. Braz. J. Biol. 67: 939-943.

Gill, T.A. 2000. Waste from processing aquatic animals and animal products: implications on aquatic animal pathogen transfer. FAO Fisheries Circular. No. 956. Rome, Italy.

Guzmán-Sáenz, F.M., Molina-Garza, Z.J., Pérez-Castañeda, R., Ibarra-Gámez, J.C. and Galavíz-Silva, L. 2009. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV) in wild shrimp (*Farfantepenaeus aztecus* Ives, 1891 and *Litopenaeus setiferus* Linnaeus, 1767) of La Laguna Madre, Gulf of Mexico. Rev. Biol. Mar. Oceanogr. 44: 663-672

Ibarra-Gámez, J.C., Galavíz-Silva, L. and Molina-Garza, Z.J. 2007. Distribution of necrotizing hepatopancreatitis bacterium (NHPB) in cultured white shrimp, *Litopenaeus vannamei*, from Mexico. Cienc. Mar. 33: 1-9.

Lightner, D.V. 1996. Disease of culture penaeid shrimp. In: Handbook of Mariculture, Crustacean Aquaculture. CRC Press. P. McVey (ed.) Boca Raton USA 393-486.

Pantoja, C.R. andLightner, D.V. 2003. Necrotizing hepatopancreatitis: diagnosis, distribution in shrimp. Global Aquaculture Advocate. 6:18.

Río-Rodríguez, R.E., Soto-Rodríguez, S., Lara-Flores, M., Cu-Escamilla, A.D. and Gómez-Solano, M.I. 2006. A necrotizing hepatopancreatitis (NHP) outbreak in a shrimp farm in Campeche, Mexico: a first case report. Aquaculture. 255: 606-609.

Vincent, A.G. and Lotz, J.M. 2007. Effect of salinity on transmission of necrotizing hepatopancreatitis bacterium (NHPB) to Kona stock *Litopenaeus vannamei*. Dis. Aquat. Org. 75: 265-268.

Wang, Q., Zhuang, Z., Denga, J. and Ye, Y., 2006. Stock enhancement and translocation of the shrimp *Penaeus chinensis* in China. Fish. Res. 80: 67-76.