Viral Infection Surveillance of Bait Shrimp in Four Texas Bays

by

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Abstract.—From August 2005 to July 2007, native bait shrimp samples were collected from four different Texas bay systems (Matagorda Bay, San Antonio Bay, Corpus Christi Bay and Lower Laguna Madre) to check for diseases of concern which may be associated with exotic farm raised and/or imported shrimp. Matagorda Bay, Corpus Christi Bay and Lower Laguna Madre all had complete samples. During the sampling period, San Antonio Bay was the only site that did not have bait shrimp consistently available. Samples were tested for Taura Syndrome Virus (TSV) and White Spot Syndrome Virus (WSSV) by collecting and preserving pleopods from each shrimp. At least 60 shrimp were used per sample. Two pleopods were collected from each shrimp and put into two separate bottles containing 95% ethanol. One pleopod sample was for TSV Polymerase Chain Reaction (PCR) testing and the other for WSSV PCR testing. All WSSV PCR tests were performed by Texas Veterinary Medical Diagnostic Laboratory (TVMDL) and TSV testing was conducted by Texas A&M University-Corpus Christi until August 2006. Afterwards, TVMDL conducted the remainder of the TSV testing as well. Overall, 87 bait shrimp samples were collected. All samples (60 shrimp per sample) tested negative for TSV and WSSV. Collecting samples from local bait stands proved to be a useful and a relatively inexpensive method to monitor shrimp health in Texas bay systems. Even though Texas farms and research facilities reported TSV and WSSV infections prior to the study period, no viruses were detected in any adjacent bay systems. The objective of the study was to test wild caught native shrimp for the Taura Syndrome Virus and the White Spot Syndrome Virus. Both of these viruses can be associated with exotic shrimp from commercial farming operations, research facilities and processing plants.

Introduction

Shrimp are the most important marine species caught in Texas and the Gulf of Mexico (GOM) in volume and associated value. In 2007 alone, the GOM yielded 80,092 metric tons of shrimp worth $306 million dollars in ex-vessel value (National Marine Fisheries Service 2007). The Texas commercial fishery yielded 12,547 metric tons of shrimp (ex-vessel value $80.8 million). In addition, Texas shrimp farmers produced 1,581 metric tons of cultured shrimp valued at $7.0 million dollars in 2007 (Texas Parks and Wildlife Department (TPWD), unpublished data). Any biological impact would have a serious effect on the Texas shrimp aquaculture industry.

Taura Syndrome Virus (TSV) has been reported to cause high mortality rates in cultured *Litopenaeus vannamei* (Pacific white shrimp), one of the most important species in shrimp aquaculture (Tang and Lightner, 1999). The virus was discovered in Ecuador in 1992 (Lightner et al. 1995; Hasson et al. 1995), which later spread to the U.S. by 1994 (Hasson et al. 1999) and to Southeast Asia by 1998 (Tu et al. 1999; Yu and Song, 2000). Ensuring TSV does not affect wild populations is essential to maintaining a healthy ecosystem.

Currently, all Texas shrimp farmers culture the exotic species *L. vannamei* in outdoor ponds and raceway systems for human food consumption. *L. vannamei* has been

an aquaculture species of choice because: 1) it has a faster growth rate in an aquaculture setting than native shrimp species; and 2) hatcheries are able to produce large numbers of Specific Pathogen Free (SPF) certified postlarvae for commercial shrimp farm operations. Since 1992, Texas shrimp farmers have only used SPF-certified Pacific white shrimp postlarvae for their pond production efforts, and various disease outbreaks have been reported in this species. TSV caused high mortalities in shrimp ponds located along the lower Texas coast during 1995 (Brock et. al. 1995; Hasson et. al. 1995 and Garza et al. 1997), and the disease spread to upper Texas coast farms afterwards being problematic through 1999 (Juan and Adami 2003). TSV re-appeared in all the lower Rio Grande Valley farms in 2004 (TPWD unpublished data).

Texas shrimp farmers also encountered another shrimp disease, White Spot Syndrome Virus (WSSV), which had been limited to Asian shrimp farms before it appeared in cultured *Penaeus setiferus* (white shrimp) in Texas and South Carolina in November 1995 (Rosenberry, 1996). Because WSSV had not been observed in the Americas prior to 1995, this virus may have been introduced from Asia where the first reported outbreaks occurred in 1992 (Durand et al. 2000). Since that time, WSSV has been responsible for high shrimp mortality rates from 80-100% (Chou et al. 1995; Nakano et al. 1998) worldwide with no viable treatment currently available to control the viral agent; therefore, shrimp farmers are restricted to management of the virus using prophylaxis measures (Briñez et al. 2003).

Viruses can spread to new geographic areas by a variety of means (Durand et al. 2000). These include the transport of infected shrimp from one site or pond to another by birds acting as vectors (Schnurrenberger et al. 1987, Garza et al. 1997, Lightner et al. 1997 and JSA 1997) and by importation and reprocessing of frozen food products (Durand et al. 2000). Frozen imported shrimp must be considered a probable source for the introduction of WSSV into the country because the United States imports thousands of tons of cultured shrimp from Asia each year (Durand et al. 2000; United States Department of Commerce 2005). Imported commodity shrimp are distributed throughout the country, and large quantities are reprocessed at shrimp packing plants in many cases near coastal areas adjacent to coastal shrimp nurseries, fishing grounds, and in some locations near shrimp farms (Environmental Protection Agency 1999). The chance of infected effluent discharge from the processing plants getting to the bays should be considered a probable source of initial infection. Shrimp bioassays have confirmed that imported frozen shrimp can convey infectious WSSV capable of producing 100% mortality in SPF *L. vannamei* culture operations (Durand et al. 2000). As early as 1995, studies indicated that *Penaeus monodon* (tiger shrimp) infected with WSSV were being sold in U.S. retail fish markets and grocery stores (Nunan et al. 1998). Specimens of *P. monodon* examined showed the characteristic appearances of small white spots on the carapace and/or a reddish discoloration, which are both signs of the white spot syndrome infection (Lightner 1996).

A major concern is that Texas marine ecosystems may be negatively impacted by discharge effluents processed from shrimp facilities. Many Texas bays directly receive wastewater or are downstream of wastewater discharges from shrimp farms, shrimp

research facilities, and/or shrimp processing plants, that could potentially transport TSV and/or WSSV infected waters. Wastewater, particularly from shrimp processing plants along the Texas coast, and the subsequent incidental use of infected bait shrimp by recreational anglers may potentially function as an avenue for disease transmission to coastal waters (Reville et al. 2005). Because of the potential threats that TSV and WSSV pose to indigenous shrimp populations, it is imperative that TPWD takes a proactive role in monitoring the health status of native shrimp. Increasing concerns about the health of indigenous shrimp and crab species and the lack of any systematic evaluation of these infectious diseases in Texas waters prompted the Texas Parks and Wildlife Department (TPWD) to initiate a survey to evaluate the general health of selected indigenous marine invertebrates along the Texas coast (Dorf et al. 2005). During the study (October 1997-September 2000) no shrimp or crab specimen sampled was determined to be infected by WSSV or TSV. Dorf et al. (2005) examined *L. setiferus* (white shrimp), *Farfantepenaeus aztecus* (brown shrimp), *F. duorarum* (pink shrimp), *Callinectes sapidus* (blue crab), and *C. similis* (lesser blue crab) in their study through histopathology and *In situ* hybridization.

However, man-power and associated expenditures required to collect samples from all nine Texas bays that are routinely monitored via the agency's long-term independent resource monitoring program are cost prohibitive. Therefore, an alternative approach would be to systematically purchase shrimp directly from bait stands. Typically, bait dealers trawl their own bait shrimp that they sell at their bait stands, or they purchase bait shrimp from local commercial shrimpers. Bait dealers are not permitted by state regulations (Section 66.007 (a)) to sell live exotic shrimp species for bait (Texas Parks and Wildlife Laws 2009).

The objective of the study was to test wild caught native shrimp for the Taura Syndrome Virus and the White Spot Syndrome Virus. Both of these viruses can be associated with exotic shrimp from commercial farming operations, research facilities and processing plants.

Materials and Methods

Bait shrimp were purchased monthly from four commercial bait stands located along the mid to lower Texas coast (Figure 1) from August 2005-July 2007. Specifically, the bait stands were located on the Lower Laguna Madre, Corpus Christi Bay, San Antonio Bay, and the Matagorda Bay complexes. Each bait stand was selected because its location was near a shrimp farm, research facility, and/or a shrimp processing plant. Most of the time (97.7%) only a single species of shrimp was available at the bait stands. All shrimp purchased at the bait stands were verified to have been collected from the immediate area of the bait stand or area of interest. At least 60 shrimp specimens were purchased for each available species on hand.

The shrimp were placed into two different 19-l containers. Each container was aerated with a battery operated Big Bubble Aerator by Marine Metal Products. Two

pleopods were extracted from each of the 60+ shrimp and put into two different 100-ml containers containing 95% ethanol. Of the two extracted pleopods per shrimp, one pleopod was put into a container for TSV (Polymerase Chain Reaction) PCR testing and the other into a container for WSSV nested PCR testing. After the pleopods were extracted, each whole shrimp was injected with Davidson's solution preservative and stored in a 3.8-l container filled with Davidson's solution. After the shrimp were in the Davidson's solution for 12-24 hours, they were transferred into 95% ethanol for histology or *In situ* hybridization examination in case the initial testing proved positive for either TSV or WSSV. During the first year of the study (August 2005-August 2006) (PCR) (a molecular-based technique consisting of repeated cycles of denaturation, annealing and extension that results in the exponential replication of a unique DNA sequence using pathogen-specific primers and Taq polymerase) was conducted for TSV testing in Dr. Joe Fox's laboratory at Texas A&M-Corpus Christi (TAMU-CC). Due to budgetary constraints, TAMU-CC could no longer perform the PCR testing free of charge to TPWD. The remainder of the TSV samples (September 2006 - July 2007) were analyzed by the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) in College Station. All testing for WSSV was conducted by the TVMDL using the nested PCR (nested PCR is a conventional PCR with a second round of amplification using a different set of primers) method. This second set of primers is specific to a sequence found within the DNA of the initial conventional PCR amplicon. The sensitivity and amount of amplicon produced is increased as a result of the second round of amplification due to reduction in any inhibitor concentrations. (Varner 2008, personal communication).

Results

During the 2 year study, TPWD collected 87 bait shrimp samples. Of the 87 total samples collected, three shrimp species were identified comprising *L. setiferus* (42), *F. aztecus* (30) and *F. duorarum* (15) (Table 1). Each sample consisted of more than 60 shrimp. This totaled to more than 5,220 specimens. Samples collected included 24 from Matagorda Bay, 13 from San Antonio Bay, 24 from Corpus Christi Bay, and 26 from the Lower Laguna Madre (Table 1). The sample size for this study was 60 shrimp per species from each bay system to determine the 5% prevalence of infection for a population greater than 100,000. The 5% prevalence of infection would mean that if 5% of the population was infected by a particular disease, 95% of the time we would be able to detect it on a sample size of 60 shrimp. In all, greater than 10,440 shrimp pleopods (5,220 pleopods each for TSV and WSSV) were extracted and examined for TSV and WSSV by PCR testing with no diseases detected.

Discussion

Our study shows findings similar to those described by Dorf et al. (2005) where 5,399 shrimp specimens randomly collected through the TPWD resource monitoring program tested negative for TSV and WSSV through histopathology and *In situ* hybridization. They have postulated that diseased shrimp may have been missed in their

study because they might have suffered disease-related mortality or could have been more susceptible to predation. In our study, samples were collected by bait shrimpers who generally trawl in specific areas where shrimp congregate (R. Saunders 2005, personal communication). If diseases were present in a population of shrimp, one would expect the common shrimp congregation area to manifest higher numbers of infected individuals. One would also expect the stressful conditions caused by crowding shrimp in live-wells would manifest any underlying infection that a shrimp may be carrying. Viruses may be present in low concentrations in wild populations without causing observable disease incidents, but aquaculture conditions (i.e., high stocking densities commonly used) may potentiate the development and spread of diseases (Joint Subcommittee on Aquaculture Shrimp Virus Work Group 1997). However, no viral diseases were detected in the present study even under conditions expected to manifest diseases caused by crowding shrimp in trawler live-wells. This study serves as a preliminary examination, and the results are encouraging because of the absence of the two viruses in any of the samples examined.

Protecting native shrimp populations from introduced disease or contaminants, we recommend periodic sampling be conducted in areas where effluent discharge waters exposed to shrimp imports are generated. A governmental certification process should be implemented for shrimp dealers and processors to ensure imported shrimp diseases are not introduced into Texas coastal waters.

The 1997-2000 study (Dorf et al. 2005) sampled shrimp and crabs, whereas this study targeted only native penaeid shrimp. Since the TPWD Shrimp Inspection Program only tests exotic shrimp, this study focused efforts on the native shrimp populations that may be susceptible to the TSV and WSSV. TPWD has no regulatory authority on incoming shrimp from other countries or to test shrimp in the processing plants. The United States Fish and Wildlife Service has regulatory authority over shipments coming from other countries, while the U.S. Department of Agriculture has regulatory authority on the processing plants. TPWD's only regulatory authority is on the aquaculture facilities that culture exotic shrimp. However, TPWD can continue to test the wild shrimp in the bays to provide information on whether these diseases possibly already exist in dormant stages until the right conditions occur or if diseases are coming in from processing plants or aquaculture operations. Recommendations would be to test shrimp at existing bait stands in all bays on a continuous monitoring basis which could be covered by the shrimp inspection program. In 1975, TPWD started sampling all bay systems for a variety of species using a variety of sampling gear to establish health of the bays (Martinez-Andrade et al. 2005). As a precautionary measure, the TPWD Shrimp Inspection Program can help with bay monitoring by sampling the bait stands on a monthly basis to determine if and when TSV or WSSV appears in the bay systems. The information generated through these studies could be useful to follow the evolution of the effects of introduced pathogens and to help design strategies and political support to reduce the impact of exotic diseases in the wild populations (Morales and Chavez-Sanchez 1999; Chavez-Sanchez et al. 2002). Required TPWD quarantine protocols for exotic species in aquaculture may have played a role in preventing shrimp related disease manifestations in Texas coastal waters.

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TABLE 1. —Sample dates and shrimp species collected from bait stand study sites along the mid to lower Texas coast (August 2005-July 2007)

Month	Matagorda Bay	San Antonio Bay	Corpus Christi Bay	Lower Laguna Madre
Aug. 2005	*L. setiferus*	*L. setiferus*	*L. setiferus*	*L. setiferus, F. aztecus*
Sep. 2005	*L. setiferus*	*L. setiferus*	*L. setiferus*	*L. setiferus*
Oct. 2005	*L. setiferus*	*L. setiferus*	*L. setiferus*	*F. aztecus*
Nov. 2005	*L. setiferus*	*L. setiferus*	*L. setiferus*	*F. aztecus*
Dec. 2005	*L. setiferus*		*F. aztecus*	*L. setiferus, F. aztecus*
Jan. 2006	*L. setiferus*		*L. setiferus*	*F. aztecus*
Feb. 2006	*L. setiferus*		*F. duorarum*	*F. duorarum*
Mar. 2006	*F. duorarum*	*F. duorarum*	*F. duorarum*	*F. duorarum*
Apr. 2006	*F. aztecus*	*F. aztecus*	*F. duorarum*	*F. aztecus*
May, 2006	*F. aztecus*	*F. aztecus*	*F. aztecus*	*F. aztecus*
Jun. 2006	*F. aztecus*	*F. aztecus*	*F. aztecus*	*L. setiferus*
Jul. 2006	*L. setiferus*		*F. aztecus*	*L. setiferus*
Aug. 2006	*L. setiferus*		*L. setiferus*	*L. setiferus*
Sep. 2006	*L. setiferus*	*L. setiferus*	*L. setiferus*	*L. setiferus*
Oct. 2006	*L. setiferus*	*L. setiferus*	*L. setiferus*	*L. setiferus*
Nov. 2006	*L. setiferus*		*F. duorarum*	*L. setiferus*
Dec. 2006	*L. setiferus*		*L. setiferus*	*L. setiferus*
Jan. 2007	*L. setiferus*		*L. setiferus*	*F. aztecus*
Feb. 2007	*L. setiferus*		*L. setiferus*	*F. aztecus*
Mar. 2007	*F. duorarum*	*F. duorarum*	*F. duorarum*	*F. duorarum*
Apr. 2007	*F. aztecus*	*F. duorarum*	*F. duorarum*	*F. duorarum*
May, 2007	*F. aztecus*		*F. aztecus*	*F. aztecus*
Jun. 2007	*F. aztecus*	*F. aztecus*	*F. aztecus*	*F. aztecus*
Jul. 2007	*F. aztecus*		*F. aztecus*	*F. aztecus*
Subtotal	24	13	24	26

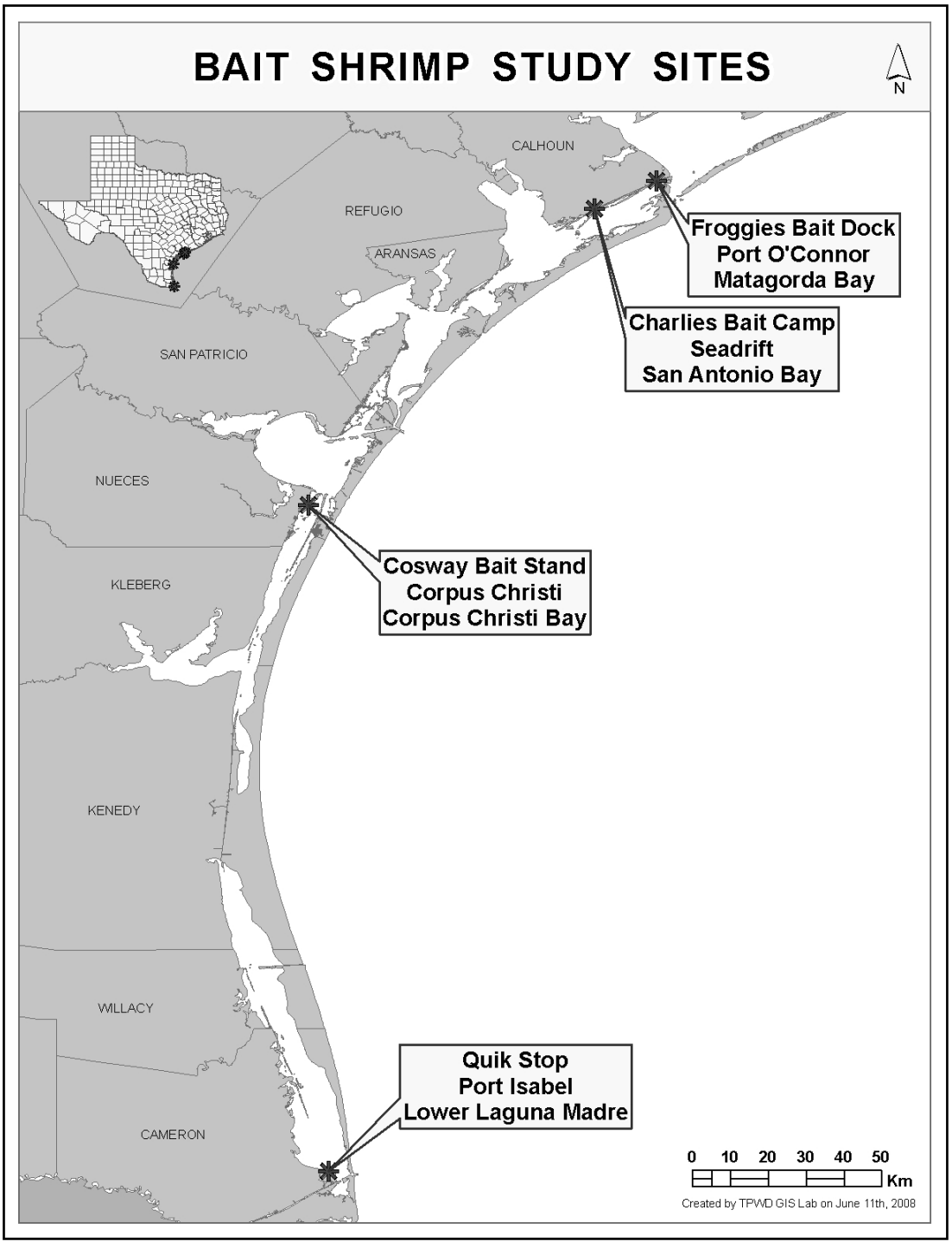

FIGURE 1. —Bait shrimp study sites along the lower to middle Texas coast.