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Susceptibility to Taura Syndrome Virus of Some Penaeid Shrimp Species Native to the Gulf of Mexico and the Southeastern United States

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Abstract

Experimental studies demonstrated that *Penaeus setiferus*, but not *Penaeus aztecus* or *Penaeus duorarum*, could be killed by Taura syndrome virus (TSV). However, specimens of *P. setiferus* that survived infection and both *P. aztecus* and *P. duorarum* at least 79 days postexposure that did not demonstrate gross signs of infection were shown to harbor virus by bioassay using *Penaeus vannamei*, a highly susceptible target host. Consequently, all three of those penaeids native to the southeast United States can serve as carriers or reservoir hosts of TSV without necessarily exhibiting disease. Infections in *P. setiferus* took longer to cause mortality than in *P. vannamei* and killed a smaller percentage of that host. Also, histological lesions diagnostic of TSV infection were not always evident in sectioned tissue of infected *P. setiferus*, and they generally were more conspicuous during Days 4–7 postexposure compared with lesions that also occurred at both earlier and later days in tissues of *P. vannamei*. Infections could be produced by injection, ingestion, and incorporation of the infective material into dietary brine shrimp. There appeared to be a difference in susceptibility to TSV disease by different stocks of *P. setiferus*, but different experiments produced conflicting evidence regarding a relationship between age and predilection to mortality. Large and small specimens of equal-aged shrimp

succumbed similarly to TSV infections for both *P. vannamei* and *P. setiferus*. The nonnative species *P. chinensis* demonstrated a high susceptibility to experimental TSV disease.

Keywords: Taura syndrome virus, TSV, *Penaeus*, shrimp, postlarva, aquaculture, susceptibility

Introduction

Economically disastrous mass mortalities of *Penaeus vannamei* caused by Taura syndrome virus (TSV) have, within the last few years, spread from South America into North America. Infections caused by this virus and its spread pose a number of serious threats, one of which concerns the ability of this virus to infect or be carried by native Gulf of Mexico and Western Atlantic Ocean penaeid shrimps. To determine whether such shrimp species can be infected or can maintain an infection was the purpose of this study.

TSV has been characterized as a picornavirus (Bonami et al., in press; Hasson et al., 1995; Lightner, 1996). Infections caused by this virus, first recognized in farms near the mouth of the Taura River, Ecuador, in June 1992, have been reviewed by Lightner et al. (1995) and Brock et al. (1995). However, the infection may have been present in Colombia at least as early as February 1990 (Laramore, referenced personal communication). In May 1994, the agent was first found in United States farms rearing *P. vannamei* on the island of Oahu, Hawaii; by October 1994, it was discovered in a maturation facility in Summerland Key, Florida; and in May 1995 it was in Texas farms. The agent has been contained in the first two cases, and its status involving its presence in Texas and elsewhere in the United States is presently uncertain. Experimentally, the virus can infect *Penaeus stylirostris*, *Penaeus schmitti*, and *Penaeus chinensis* (see Lightner, 1996; this study). In this study, we test the susceptibility of *Penaeus setiferus*, *Penaeus aztecus*, and *Penaeus duorarum* to infection by TSV as well as the ability of the native shrimps to carry the agent. We consider the term "susceptible" to mean that the shrimp will permit an infection of TSV to become established, not necessarily producing a disease state. As the pathogenic effect of the infection increased, it could cause disease with or without clinical signs. Disease in some hosts and under some conditions could cause death.

Materials and Methods

Experimental Animals/Virus

Two independent series of bioassays were conducted, one in facilities at Gulf Coast Research Laboratory (GCRL) and the other at University of Arizona (UA). For some of these bioassays, specimens of the three native shrimp species studied were produced at Waddell Mariculture Center (WMC), South Carolina Department of Natural Resources. These were sent to GCRL and UA, both on 7 June 1995 as postlarvae: *P. setiferus* [PL 9 (a term used to indicate that the shrimp had been postlarvae for 9 days), 19 total days in culture, from >2 female broodstock], *P. aztecus* (PL 12, 24 days in culture, from 5 females), and *P. duorarum* (PL 13, 21 days in culture, from 1 female). In the strict sense, postlarvae could probably be considered young juveniles when reaching about PL 17, but, nevertheless, we use the PL

convention with a date for older shrimp to designate the age of the shrimp following metamorphosis from Mysis 3 even though they may no longer be postlarvae. The broodstock for postlarvae of all three species were captured wild off South Carolina; the broodstock of *P. setiferus* was collected from two different locations: near Hilton Head Plantation, N 32°-12′-65″, W 80°-38′-84″, and near Charleston off Morris Island, N 32°-43′-30″, W 79°-50′-80″. All these postlarvae were held for about 1 week before testing, in 208-liter aquaria at GCRL and in 1500-liter tanks at UA. Individuals of these shrimp stocks were used first to test as late postlarvae, and then other individuals were grown out to use as juveniles for further testing.

Specimens of *P. setiferus* from additional sources were also used in bioassays. Reared specimens were received as PL 10s on 28 June 1995 from a commercial producer in South Texas and used as PL 33s. Wild juvenile specimens from Davis Bayou, Ocean Springs, Mississippi, were estimated to be a little more than a month old on 5 July 1995 when collected. They were held for 16 days before the test was initiated, and they survived well during that period, as did the controls during the test. Additional specimens (about 1 year old, but not reproductively mature) were collected from the same area in March 1996 and held to conduct tests.

For studies at UA using subadult shrimp, wild specimens of the brown shrimp *P. aztecus* (7.68 g av wt) were provided by Texas A&M, hatchery-produced specimens of *P. setiferus* (3.5 g av wt) were obtained from a commercial hatchery in Texas, and postlarvae of *P. chinensis* (2.1 g av wt) produced in a hatchery in Korea were shipped to Arizona.

Specimens of *P. vannamei* used as positive and negative controls were either high health status stock, originating directly or indirectly from specific pathogen-free (SPF) broodstock or from shrimp in tertiary quarantine (Lotz et al., 1995; Pruder et al., 1995), involved in the U.S. Marine Shrimp Farming Program (USMSFP). Some were produced at GCRL either from broodstock received from The Oceanic Institute (OI), Makapuu Point, Oahu, Hawaii, or from offspring of wild Guatemalan broodstock which had undergone quarantine at GCRL. Others were produced at WMC, with SPF broodstock (Kona) sent from Keahuolu (USMSFP nucleus breeding facility) (District of Kona), Hawaii. UA used SPF *P. vannamei* from Kona, Hawaii, as controls. None were known to be infected with any virus species.

Whereas the identity of most specimens of the four principal species that we studied could be readily determined on the basis of morphological features of the specimens and of the broodstock, some representative postlarvae and juveniles also were assessed by Kenneth Stuck at GCRL with isoelectric focusing. We confirmed that the species in all populations that we considered to be *P. setiferus* was *P. setiferus* and what we considered *P. vannamei* was *P. vannamei*. *Penaeus setiferus*, *P. vannamei*, *P. aztecus*, and *P. duorarum* could all be differentiated biochemically and morphologically. Voucher specimens are being maintained by Stuck.

Dietary food for shrimp consisted of commercial pellets (Rangen No. 2 and No. 4) and brine shrimp. After initial administration of the infective TS agent at both facilities and on alternate days when not administered infective tissues at UA, shrimp were fed on a diet of commercial pellets. Shrimps were typically fed early in the morning and near noon. At GCRL, the diet for postlarvae was often supplemented with recently hatched brine shrimp (Aquarium Products, Lot No. 756) during the noon feeding. Based on data from negative

controls and from other studies not reported here, there was no indication that TSV or other viruses were present in the diets, pellets, or brine shrimp. No antibiotics were used in any of the experiments.

The TSV-infected tissues used in nearly all the studies originated from a single epizootic in *P. vannamei* from southern Texas during early May 1995, even though that material used by GCRL and UA came from infected shrimp at two different farms. At GCRL, the original material from the farm had been passed twice through *P. vannamei* and then frozen at –70°C before being used in all the experiments reported here from GCRL. At UA, infected material from the farm was frozen and used when needed. All material produced the same histopathological alterations, appeared to have the same virulence, and was considered identical. Separate material used for injecting subadults was a sucrose gradient-purified, Ecuadorian isolate of TSV collected in 1993.

The virus was administered differently for specific tests: *per os*, incorporated into brine shrimp, and injected intramuscularly. At present, no adequate way exists to estimate the actual number of virions administered to a shrimp. Nevertheless, all TSV samples consisted of a portion of 10 to 20 different whole individuals of known TSV-infected stock mixed randomly together. For direct exposures, experimental groups were exposed to this infected material diced, usually on the basis of 5% body weight. For those groups exposed to virus in brine shrimp, we allowed them to feed on 48-hr-old brine shrimp that previously had been allowed to feed on the minced TSV-infected tissue in a well-aerated Erlenmeyer flask for 2 hr or longer (Overstreet et al., 1988). The exposed groups were allowed to feed twice on the brine shrimp, once in the morning and once about noon. Usually the excess slurry of infected tissue not incorporated into the brine shrimp was administered with the brine shrimp during the second feeding. However, at least when incorporating baculovirus into brine shrimp, all exposed penaeid shrimp in a group become infected, even with one feeding (Overstreet, 1994). For injections at GCRL, a mixture of the TSV-infected material was weighed and mixed with 1 ml 0.8% Ringer's physiological saline (autoclaved) per gram of tissue. This material was then blended, minced with mortar and pestle, and finally ground with a cell tissue grinder. This slurry was centrifuged for 5–15 min at 2700 rpm, and the supernate filtered through a 0.2-μm syringe filter. This filtrate containing virus was then diluted to 1 part filtrate:7 (or 8–10) parts physiological saline. Unless diluted, this filtrate, whether from infected or uninfected shrimp, was toxic to shrimp. About 0.01–0.05 ml of this TSV-containing fluid, depending on the size of the bioassay shrimp, was then injected intramuscularly into the abdominal muscles (tail) of juvenile to adult specimens. For intramuscular injection of subadults at UA, 0.02 ml of sucrose gradient-purified Ecuadorian TSV that had been diluted 1:100 with 2% NaCl was injected intramuscularly into the third tail segment of shrimp in one experiment with a corresponding sucrose gradient-purified inoculum prepared from TSV-free tissue from SPF *P. vannamei* and injected into negative controls. In the other experiment, 0.02 ml of TSV inoculum was injected into each of the *P. vannamei* positive controls, and 0.08 ml was injected into the much larger individuals of *P. aztecus*, while a corresponding volume of sterile 2% NaCl solution was injected into the negative controls. In tests at both facilities, all injection treat-

ments for experimental and sham control shrimp consisted of a single injection. The purpose of this study was not to assess dose, but doses administered were always considered by us in excess of what was necessary to cause mortality in susceptible individuals.

Histological Evaluation

For histological evaluation with light microscopy, both routine samples and moribund individual shrimp were liberally injected with Davidson's fixative, and routine procedures were followed for preparation, sectioning, and staining with hematoxylin and phloxine/eosin (H&E) (Bell and Lightner, 1988). Sections were examined for characteristic TSV lesions. Such lesions consist of multifocal areas of acute necrosis of the cuticular epithelium and subcutis of the exoskeleton, gills, appendages, foregut, and hindgut. Lesions contain karyorrhectic nuclei and numerous variably sized eosinophilic to basophilic cytoplasmic inclusion bodies, giving them a characteristic "peppered" or "buckshot" appearance (Lightner et al., 1995).

Representative postlarval specimens of *P. setiferus* and *P. aztecus*, as well as positive controls of *P. vannamei* with pathodiagnostic TS lesions in H&E preparations, were tested for TSV using a nonradioactive, DIG-labeled cDNA probe for TSV in *in situ* hybridization assays (Lightner, 1996).

Facilities and Experimental Aquaria

Studies were conducted at both the GCRL and UA. They took place in isolated rooms with footbaths containing [Wescodyne, a 1.6% titratable iodine detergent-germicide or calcium hypochlorite (LeBlanc and Overstreet, 1991)] at each door's entrance. Personnel not involved directly with the tests were prohibited from the areas containing infected shrimp.

Bioassays of specimens from the same or similar penaeid stocks of different ages as well as specimens from other sources were conducted typically in 38-liter aquaria in closed systems. Aquaria at GCRL were fitted with dual aerated sponge filters, conditioned with Fritz-zyme No. 9 (nitrifying bacteria), covered with glass covers, and provided with separate dip nets. They were kept under continuous light, and most were placed in a 27 ± 1°C water bath. Others were maintained in a room with relatively constant ambient temperature about 25–26°C, with the temperature of representative aquaria monitored twice daily. Salt water was prepared with unchlorinated well water and hw-MARINEMIX + Bio-Elements, except for a few cases, when Forty Fathoms Bio-crystals Marinemix was used to prepare salt water for experiments with late juveniles or subadults. Those aquaria at UA were covered by a plastic sheet, and each had an individual airlift system with an oyster biofilter and aerator. Salt water was prepared with Forty Fathoms Bio-crystals Marinemix. At both facilities, representative aquaria were tested every day or two for nitrate (to keep within 0.25–1.0 ppm), ammonia (to keep within 0–0.5 ppm), and temperature (maintained at 25–27°C). Water was not exchanged or modified unless values exceeded those values, and that was seldom. In the few cases when nitrogen limits were exceeded, water was exchanged. This water and water in aquaria at the end of tests was disinfected first with the iodine solution and then left standing with about 120 ml of commercial bleach per water-filled 38-liter aquarium.

Experimental Design

The initial experiments were conducted with postlarvae to assess whether the three native penaeid species (*P. setiferus*, *P. aztecus*, and *P. duorarum*) were susceptible to TSV infection and to determine the pathogenicity of infection. At GCRL, a total of 30 shrimp was placed in each aquarium. There were duplicated aquaria for each of the five exposed groups plus two more for each of the nonfed controls, making a total of 20 aquaria: *P. setiferus* (PL 15), *P. aztecus* (PL 18), *P. duorarum* (PL 19), *P. vannamei* (HI, PL 77 + PL 78), and *P. vannamei* (GCRL, PL 64 + PL 71). The aquaria were maintained in a 27°C water bath except for a few containing control groups, which were maintained at room temperature of 24–26°C (usually 26°C); all aquaria had a salinity of 28 to 30 ppt.

TSV was administered to each replicate experimental group in both a morning and a noon feeding of brine shrimp with incorporated TSV-infected material. Negative control groups were allowed to feed on nonexposed brine shrimp. All the aquaria were kept in the same area and examined for the status of individual shrimp three or four times a day. About five bioassay shrimp from each aquarium were fixed in Davidson's fixative to confirm the infection histologically.

Experimental conditions in aquaria where shrimp did not all die or serve as histological samples were continued for 79 days. At that time, most of those remaining shrimp were frozen at –70°C to determine by bioassay if they carried TSV. Also, a few shrimp were fixed near the beginning and at the termination of the experiment for histological evaluation. Dead shrimp were left in the aquaria, allowing others to feed on them and reduce opportunities for contamination.

After the same stock of *P. setiferus* from WMC reached PL 25, groups were similarly tested again to confirm the ability of *P. setiferus* to become infected and to assess the factor of age on susceptibility. There were also 30 shrimp per 38-liter aquarium, and those with *P. setiferus* but not *P. vannamei* were duplicated for a total of six aquaria, including a nonexposed control group for each treatment. Shrimp were maintained in aquaria with 28 ppt salinity at 26–28°C. The same stock of TSV was administered to the penaeids in brine shrimp as described above. Moribund shrimp and a sample of three were fixed for histologic confirmation of TSV.

In a simultaneous independent study, UA exposed the same stocks of shrimp to TSV to determine the histological aspects of infections. Two feedings of 0.6 g infected minced tissue fed directly without incorporation into brine shrimp were presented to shrimp on Days 0, 2, 4, and 6 in a 15-day study. There were approximately 200 postlarvae introduced per aquarium, with one exposed and one negative control group for each species: *P. setiferus* (PL 9), *P. aztecus* (PL 12), *P. duorarum* (PL 13), and *P. vannamei* (0.13 g av wt, with only 25 individuals per aquarium). The control aquaria were physically isolated from experimental ones. Salinities in aquaria with native shrimp averaged 30 ppt, and those with *P. vannamei* averaged 20 ppt. The health of shrimp was observed four times a day, two during feedings. For histological evaluation, 5–10 individuals per tank were sampled periodically at 7 AM from Day 0 to the termination of tests at Day 15 or until a group underwent an epizootic, and these samples along with moribund individuals were fixed for histological evaluation. Positive control *P. vannamei* were sampled when moribund on Days 3 and 4 and then the remainder of shrimp on Day 5 when signs of TSV infection were present.

After 38 days, another experiment at GCRL with the same and additional stocks of shrimp was initiated to determine if age, size, or origin of *P. setiferus* had an obvious influence on mortalities caused by TSV. The group of *P. setiferus* PL 53s as well as an older group of *P. vannamei* (PL 86 + PL 88) produced at WMC with USMSFP Population 1 broodstock were both graded on size into relatively large and relatively small individuals. Isolated groups of 60 large and 60 small shrimp were then each divided into two equal groups of 30, one to serve as an experimental group administered TSV in brine shrimp and one to serve as a negative control group. For positive controls, groups of relatively old individuals (PL 86 and 88) of *P. vannamei* from the same spawn were similarly sorted into large (3 g) and small (1.5 g) specimens, with groups of each used for either injecting with TSV or feeding TSV in brine shrimp. There was a corresponding nonexposed control for each group. The experiment also included TSV-fed PL 33 of *P. setiferus* produced commercially in a Texas hatchery and some both TSV-fed and TSV-injected wild specimens caught in Mississippi. Additional juvenile *P. vannamei* to be used as positive controls were produced at GCRL with high health status broodstock provided by OI. These bioassays were conducted in 38-liter aquaria with 28–30 ppt water at 25–26° or 27°C, if in a water bath. Representative shrimp were examined for histological confirmation of TSV.

As a continuation of studies to assess older juveniles, an additional study at GCRL on the Texas stock of *P. setiferus* was also conducted when members were PL 51; individuals in exposed groups were fed 5% body weight (i.e., 0.5 g for *P. setiferus*) of TSV-infected tissue per aquarium. The replicated groups of 100 *P. setiferus* and 2-g *P. vannamei* controls were maintained in 38-liter aquaria, while 12-g *P. vannamei* controls were maintained in 114-liter aquaria. Aquaria were maintained as indicated above. Number of surviving individuals was determined at Day 13. Moribund specimens were evaluated histologically for TSV lesions, and the surviving shrimp at Day 14 were frozen at –70°C to bioassay as potential carriers. An independent study at UA was conducted on older juveniles of the identical three native stocks, using *P. vannamei* as controls. Each of the four treatment groups were fed 1.4 g of minced, TSV-infected tissue on Days 0 and 1 and 2.8 g/day on Days 2, 3, and 4. Pelleted ration was administered *ad lib.* thereafter until the experiment was terminated on Day 10. All control shrimp were maintained on the same pelleted ration (1 g/tank/day) for the duration of the experiment. Aquaria were maintained with salinities ranging from 25 to 26 ppt and temperatures ranging from 26 to 30°C, with a mean of 28°C.

To assess the possibility that subadults of *P. setiferus* were more resistant to mortality from TSV than *P. setiferus* from different stocks and other species, we injected different groups with TSV at both facilities. UA injected sucrose gradient-purified TSV (Ecuadorian isolate) intramuscularly into 10 specimens of *P. setiferus* (3.5 g), *P. aztecus* (7.7 g), and *P. chinensis* (2.1 g), as well as 15 specimens of *P. vannamei* (2.1 and 1.8 g) as positive controls. Similar groups were used as negative controls for all species. The different species were maintained in 90-liter aquaria with different conditions (i.e., 24 ppt/27°C, 33 ppt/28°C, and 23 ppt/26°C for the test groups of *P. setiferus*, *P. aztecus*, and *P. chinensis*, respectively, and 24 ppt/25°C and 21 ppt/28°C for *P. vannamei*). The assay with *P. aztecus* was not conducted concurrently with the others, and it used negative controls injected with saline rather than extract from noninfected shrimp tissue. Effort was directed to obtaining data on when exposed shrimp died and to verifying TSV infection histologically. At GCRL, we collected

local subadult wild individuals of *P. setiferus* from Davis Bayou, Mississippi, maintained them for about 2 weeks, transferred them to 76-liter aquaria, and then injected them in the tail (between the second and third segments) with a filtrate as described above. Shrimp were maintained in 15 ppt seawater at 24–26°C, with water quality measured every third day. Representative specimens were examined histologically to confirm TSV.

A final experiment was conducted at GCRL to determine if infective TSV was still present in survivors of the previously described experiments with *P. setiferus*. We also tested tissues from *P. aztecus* and *P. duorarum* frozen on Day 79 of the first experiment. As indicated before, the survivors from the previous experiments were frozen at –70°C for this experiment. Minced material from a portion of several individuals from these sources was either incorporated into brine shrimp to feed shrimp or prepared as a 1:8 dilution of filtered supernate to inject into shrimp. In all cases, *P. vannamei* was used as the bioassay animal. There were two different sources of *P. vannamei*, relatively large ones (PL 153, 154) produced at GCRL from OI high health status stock (Case numbers CDL-9 501-1&2) and smaller ones (PL 91) produced at WMC from 23 different females (Kona broodstock). Because of the nature of the experiment, there were no additional positive controls. Aquaria for all groups had a salinity of 28–30 ppt maintained at 26–27°C, and the shrimp were fed pellets and brine shrimp. Deaths were observed two to four times per day, and the number of survivors was recorded on Days 6 and 10. Moribund specimens and a few random specimens at the termination of the study were fixed with Davidson's fixative for histological evaluation.

Results

Table 1 shows that *P. setiferus* (PL 15) was as susceptible to TSV infection as were two different populations of somewhat older *P. vannamei* positive control groups (PL 64–78). There was as much difference in the number of days it took for the first shrimp in a test group to die from infection and the number of days when most shrimp died between replicate aquaria for each of the two shrimp species as there was between those periods when comparing values for the two species. In the corroborating experiment conducted 10 days after the first one, results were similar except that most specimens of *P. setiferus* (initially PL 25) died a few days later than the *P. vannamei* positive controls. The presence of TSV based on histological demonstration of characteristic lesions in representative specimens of *P. setiferus* (including as late as Day 9) and *P. vannamei* confirms that the dying shrimp were infected. Table 2 shows that those clinical signs in mild to severe cases were most common in shrimp from Days 4 to 7 and rarely seen in later samples. Moreover, no evidence supported an infection that resulted in mortality of either *P. aztecus* or *P. duorarum* as was demonstrated in *P. setiferus*. Even though none of the few individuals of *P. aztecus* and *P. duorarum* examined histologically with H&E preparations demonstrated conclusive TSV lesions, a few small lesions suggestive of TS were observed in a few exposed individuals of both those species. However, lesions characteristic of TSV from representative specimens of *P. setiferus*, *P. aztecus*, and *P. vannamei* were confirmed as TSV by gene probe diagnosis using *in situ* hybridization assays. Time-sequenced histological data for a later study (Table 2) showed that 25% (3 of 12) of the Day 3 sample of *P. aztecus* had typical

histological lesions of TSV infection. Neither samples of *P. aztecus* after 3 days nor any samples of *P. duorarum* revealed the same such conclusive lesions. Moreover, in the infections in *P. setiferus*, there was no relationship between severity of lesions and length or time of infection from Days 3 to 9. However, by Days 6 and 7, some indication of infection was obvious histologically in most of the samples of *P. setiferus* compared to earlier histological indications on Day 4 in samples of *P. vannamei*.

Table 1. Exposure of Replicate Groups of 30 Young Individuals of *Penaeus setiferus*, *Penaeus aztecus*, and *Penaeus duorarum* Produced in a Hatchery from Wild Broodstock in South Carolina to Taura Syndrome Virus (TSV)–Infected Tissue Incorporated into Brine Shrimp

Group[a]	Initial age of shrimp	Day of initial deaths	Days when most died	Day when remainder died	No. dead by Day 11	Deaths probably non-TSV related
Exposed *P. setiferus*	PL 15	3, 5	5, 7	7, 7	30, 30	
Nonexposed *P. setiferus*	PL 15	—, —[b]	—, —	—, —	1, 0	1 on Day 7
Exposed *P. aztecus*[c]	PL 18	—, —	—, —	—, —	1, 0	1 on Day 4
Nonexposed *P. aztecus*	PL 18	—, —	—, —	—, —	0, 0	
Exposed *P. duorarum*[c]	PL 19	—, —	—, —	—, —	0, 0	
Nonexposed *P. duorarum*	PL 19	—, —	—, —	—, —	0, 0	
Exposed *P. vannamei*	PL 77, 78	2, 2	4, 6	4, 8	30, 30	
Nonexposed *P. vannamei*	PL 77, 78	—, —	—, —	—, —	0, 0	
Exposed *P. vannamei*	PL 64, 71	2, 3	3, 6	4, 8	30, 30	
Nonexposed *P. vannamei*	PL 64, 71	—, —	—, —	—, —	0, 0	
Exposed *P. setiferus*[d]	PL 25	2, 8	10, 13	13, 17	15, 3	
Nonexposed *P. setiferus*[d]	PL 25	—, —	—, —	—, —	1, 0	1 on Day 7
Exposed *P. vannamei*[d]	PL 87, 88	2	3	—	30	
Nonexposed *P. vannamei*[d]	PL 87, 88	—, —	—, —	—, —	0	

a. Tests were conducted with *Penaeus vannamei* as positive and negative controls, with paired values indicating values corresponding to data for each replicate aquarium. Sources of *P. vannamei:* produced at GCRL either from high health status (free of at least all disease agents being investigated) broodstock received from The Oceanic Institute, Hawaii (PL 78, 77 and 87, 88 [PL 78 is a convention describing a shrimp that is in its 78th day after metamorphosing into a postlarva]), or from offspring of wild Guatemalan broodstock which had undergone quarantine at GCRL (PL 64, 71).

b. (—) None observed.

c. Specimens of *P. aztecus* and *P. duorarum* remaining at Day 79 were frozen to bioassay as carriers of TSV.

d. To confirm the mortalities of *P. setiferus*, these groups were tested 10 days later with the same stock of *P. setiferus*, stock of virus, and conditions.

Table 2. Exposure of Young Individuals of *Penaeus setiferus*, *Penaeus aztecus*, and *Penaeus duorarum* by Direct *per os* Feeding on TSV-Infected Tissue Demonstrating Histological Severity and Prevalence of Infection

	No. sampled at sample day[b]									
Group[a]	0	3	4	6	7	9	12	15	No. of shrimp demonstrating histological TSV-lesions[c]	Severity of infection
P. setiferus exposed	0	10	1	13	4	16[d]			1, 1, 6, 4, 2	Mild to severe
P. setiferus not exposed	25	6	0	10	0	10	0	10	0, 0, —, —, —[e]	Negative
P. aztecus exposed	0	12	0	10	0	10	5	10	3, 0, 0, 0, 0	Very mild to severe
P. aztecus not exposed	25	6	0	10	0	10	0	15	—, 0, —, —, —[e]	Negative
P. duorarum exposed	0	10	0	10	0	10	5	10	0, 0, 0, 0, 0	Negative
P. duorarum not exposed	25	6	0	10	0	10	0	10	—, 0, —, —, —[e]	Negative
P. vannamei exposed	0	1	6	7[f]					1, 6, 3	Mild to severe
P. vannamei not exposed	5	0	0	0	0	0	0	17	0, —[e]	Negative

a. Tests were conducted for 15 days, initially with approximately 200 individuals per aquaria for all but the groups of *Penaeus vannamei* serving as positive and negative controls.
b. The numbers in the top row represent the day of the study, and those in the remaining rows below represent the number sampled at each of the above corresponding days.
c. Values refer to corresponding number of shrimp with lesions out of the sample number listed in column to left. Representative specimens of *P. setiferus*, *P. aztecus*, and *P. vannamei* with lesions characteristic of TSV were confirmed by gene probe diagnosis using *in situ* hybridization assays.
d. Early termination because remaining shrimp died.
e. Because of the lack of mortalities, most of controls were not processed as indicated by "—."
f. Early termination at Day 5.

Since *P. setiferus* could become infected with TSV and die, we investigated the relationship among mortality, age, size, and origin of stock (Table 3). Groups of select, relatively small individuals of the same age for both *P. setiferus* and *P. vannamei* demonstrated a few more mortalities occurring a short time period before being observed in their larger counterparts; however, we do not consider the differences biologically significant. Notable, however, were the later and fewer mortalities observed for *P. setiferus* compared with those for *P. vannamei*. Moreover, the stock of *P. setiferus* from Texas appeared to be less susceptible to TSV than those from other locations. None of the exposed Texas shrimp died within the first 11 days, a period when most shrimp from the other localities died. In this experiment, little difference occurred in the effect of TSV on juvenile shrimp when exposed by injection or by ingestion.

Table 3. Dynamics of Taura Syndrome Virus Infections in Juvenile *Penaeus setiferus* Relative to Size and Age of Shrimp, to Origin of Shrimp, and to Method of Exposure

Group[a]	Age of shrimp at start of test	*N*	Average total length/weight at Day 17 of experiment	Day of initial deaths	Days of high No. deaths	No. died by Day 11
Exposed fed large SC *P. setiferus*	PL 53	30	36.2 mm/0.304 g	7	11	7
Control large SC *P. setiferus*	PL 53	30	36.2 mm/0.304 g	—	—	—[b]
Exposed fed small SC *P. setiferus*	PL 53	30	31.8 mm/0.262 g	8	8	6
Control small SC *P. setiferus*	PL 53	30	31.8 mm/0.262 g	— (7)	—	—[b]
Exposed fed Texas *P. setiferus*	PL 33	30	20.5 mm/0.072 g	19	19	0
Control Texas *P. setiferus*	PL 33	30	20.5 mm/0.072 g	—	—	—[b]
Exposed fed *P. vannamei*	PL 115, 116	30	27.7 mm/0.159 g	4	5	30
Control *P. vannamei*	PL 115, 116	30	27.7 mm/0.159 g	—	—	—[b]
Exposed fed MS *P. setiferus*	Wild	15	2.5 g	9		3
Control MS *P. setiferus*	Wild	15	2.5 g	—	—	—[b]
Exposed injected MS *P. setiferus*	Wild	15	2.5 g	1[b]		7
Control injected MS *P. setiferus*	Wild	15	2.5 g	—	—	—[b]
Exposed fed SC *P. vannamei* large	PL 86, 88	15	3 g	2	5	15[b]
Control fed SC *P. vannamei* large	PL 86, 88	15	3 g	—	—	—[b]
Exposed injected SC *P. vannamei* large	PL 86, 88	15	3 g	1[b]	3	15[b]
Control injected SC *P. vannamei* large	PL 86, 88	15	3 g	—	—	—[b]
Exposed fed SC *P. vannamei* small	PL 86, 88	15	1.5 g	2	4	13[b]
Control fed SC *P. vannamei* small	PL 86, 88	15	1.5 g	—	—	—[b]
Exposed injected SC *P. vannamei* small	PL 86, 88	15	1.5 g	1[b]	3	15[b]
Control injected SC *P. vannamei* small	PL 86, 88	15	1.5 g	—	—	—[b]

a. Source of *P. vannamei:* produced at GCRL from high health status broodstock received from The Oceanic Institute, Hawaii (PL 115, 116) or produced at Waddell Mariculture Center (WMC), South Carolina Department of Natural Resources, with 23 female USMSFP SPF broodstock sent from Kona, Hawaii (1.5 and 3 g).

b. A few sampled for histological evaluation.

Additional specimens (PL 51) from the same seemingly resistant Texas stock of *P. setiferus* were allowed to feed directly on TSV-infected tissue, and about 70% of the shrimp in each of two replicate aquaria with those died from the infection (Table 4). That mortality in that group was greater than that noted for the much older *P. vannamei* positive controls. The later bioassay not only demonstrated that the stock from Texas was not always resistant, but it also corroborated findings indicated above that older individuals from the same stock were not necessarily more resistant to the effects of TSV than younger ones.

Table 4. Survival of Young Juvenile (PL 51) *P. setiferus* 13 Days after Feeding on 0.5 g of Taura Syndrome Virus-Infected Tissue per Aquarium

Group[a]	Weight (in g)	*N*	Survival at Day 13
TX *P. setiferus*[b]	0.1	100	28%
TX *P. setiferus* replicate[b]	0.1	100	32%
Positive control *P. vannamei*	2	20	60%
Negative control *P. vannamei*	2	20	100%
Positive control *P. vannamei*	12	15	53%
Negative control *P. vannamei*	12	15	100%

a. *P. vannamei* controls were produced at GCRL.
b. Survivors were frozen to bioassay as carriers.

In contrast with the GCRL results of testing older specimens of *P. setiferus* and other species, Table 5 illustrates that a test at UA conducted in a different manner with the identical South Carolina stocks did not produce mortalities in the older specimens of *P. setiferus.* Neither did it produce mortalities of *P. aztecus* or *P. duorarum*. Moribund shrimp were observed in the *P. vannamei* treatment group only, but they were not examined histologically because of the observed clinical signs typical of TSV infections and the lack of mortalities in the corresponding control tank. When older specimens of *P. setiferus* from Texas were injected with the same amount of purified TSV as slightly smaller *P. chinensis* and *P. vannamei*, they neither died nor revealed histological lesions of infection. Neither did infections become evident in wild specimens of *P. aztecus* given a larger but relatively similar (volume to weight) dose (Table 6). On the other hand, subadult wild specimens of *P. setiferus* from Mississippi demonstrated deaths consistent with TSV-caused mortality (Table 7). There were some unexplainable mortalities of negative controls restricted to Day 5, 3 days after most TSV-injected shrimp started dying and when most had died. Both experimental and negative control groups of these wild shrimp had an infection of apostome ciliates in the gills. In assays at both UA and GCRL, TSV-exposed moribund *P. vannamei* displayed clinical signs characteristic of TSV infection, confirmed by histological examination (Tables 6 and 7).

Table 5. Daily and Cumulative Percent Mortality Results of TSV Exposed *P. vannamei*, *P. setiferus*, *P. aztecus*, and *P. duorarum* Juveniles

Group[a]	Mean weight per individual (in g)	Day											Cumulative mortality on Day 10 (%)
		0	1	2	3	4	5	6	7	8	9	10	
Exposed *P. vannamei*	1.0	0	0	1	5	3	1	2	0	0	0	0	12(80)
Control *P. vannamei*	1.0	0	0	0	0	0	0	0	0	0	0	0	0(0)
Exposed *P. setiferus*	1.1	0	0	0	1	0	0	0	0	0	0	0	1(7)
Control *P. setiferus*	1.1	0	1	0	0	0	1	0	0	0	0	0	2(13)
Exposed *P. aztecus*	0.6	0	0	0	0	0	0	0	0	0	0	0	0(0)
Control *P. aztecus*	0.6	0	0	0	0	0	0	0	0	0	0	0	0(0)
Exposed *P. duorarum*	0.8	0	0	0	0	0	0	0	0	0	0	0	0(0)
Control *P. duorarum*	0.8	0	0	0	0	0	0	0	0	0	0	0	0(0)

a. The test shrimp in 15 per group were allowed to feed directly on minced TSV-infected tissue for a 5-day period.

Histopathological evaluation of *P. setiferus* revealed the variably sized eosinophilic and basophilic cytoplasmic inclusion bodies in cuticular epithelium of the cuticle, gills, appendages, foregut, and hindgut (Figs. 1–7). Similar lesions were also present in the abdominal musculature of a few individuals (Figs. 8 and 9). Figures 10 and 11 show the reaction of the DIG-labeled cDNA probe to TSV in an appendage and the hindgut.

Table 6. Exposure by Intramuscular Injection of a Purified Viral Suspension of TSV of Subadult Individuals of *P. setiferus*, *P. aztecus*, and *P. chinensis* with *P. vannamei* as a Control, Showing Daily Mortalities and Histological Evidence of Infection

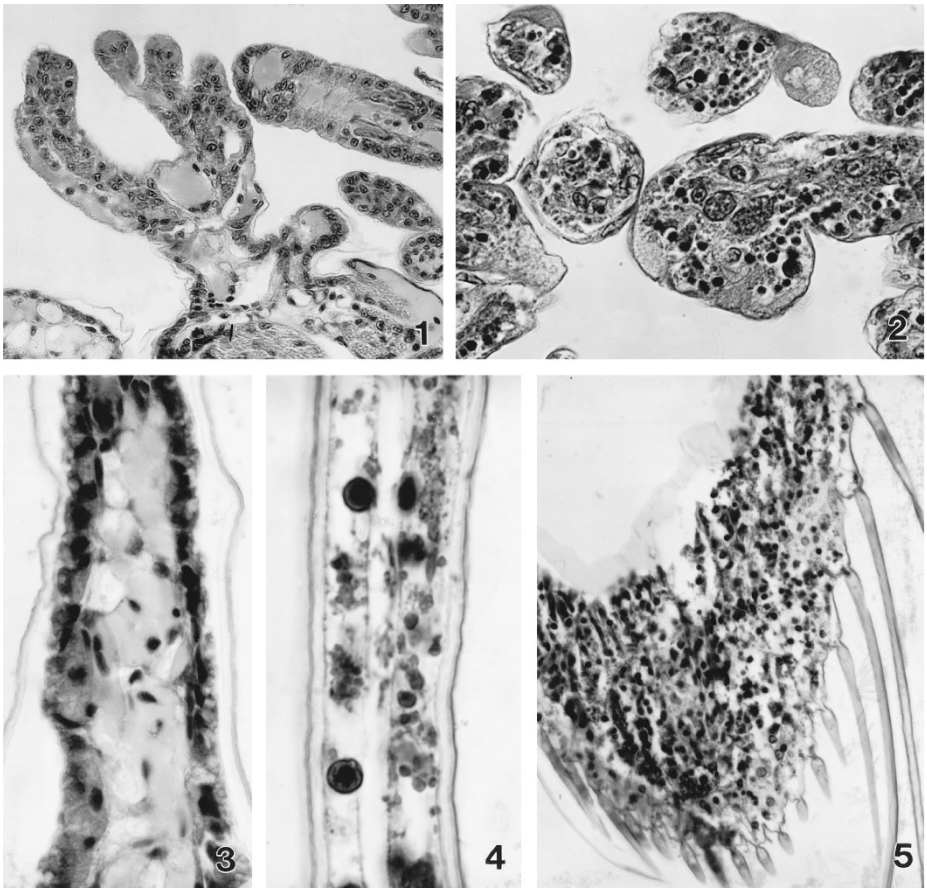
Group[a,b]	Av wt (in g)	Daily mortalities 0	1	2	3	4	5	6	7	8	9	10	Survivors of *N*	Number with TSV lesions per histologically sampled (day sampled)	Severity of infection
P. setiferus exposed	3.5	0	0	0	0	0	0	0	0	0	0	0	10 of 10	0/1 (3), 0/1 (5), 0/8 (10)	Negative
P. setiferus not exposed[c]	3.5	0	0	0	0	0	0	0	0	0	0	0	10 of 10	0/6 (0), 0/4 (10)	Negative
P. chinensis exposed	2.1	0	3	2	1	0	1	—	—	—	—	—	3 of 10	1/1 (1), 1/1 (2), 0/3 (5)	Very mild
P. chinensis not exposed[c]	2.1	0	0	0	0	0	0	—	—	—	—	—	10 of 10	0/6 (0)	Negative
P. vannamei exposed	2.1	0	4	4	3	2	0	—	—	—	—	—	2 of 15	3/3 (1), 3/3 (2), 1/1 (3), 0/2 (5)	Mild to severe
P. vannamei not exposed[c]	2.1	0	0	0	0	0	0	—	—	—	—	—	20 of 20	0/10 (0), 0/10 (5)	Negative
P. aztecus exposed	7.68	0	0	0	0	0	0	0	0	0	0	0	10 of 10	0/2(5), np[d]/8 (10)	Negative
P. aztecus not exposed[e]	7.68	0	0	0	0	0	0	0	0	0	0	0	11 of 11	np/6 (0), 0/5 (10)	Negative
P. vannamei exposed	1.84	0	3	6	0	0	1	—	—	—	—	—	5 of 15	np/1 (1), 2/2 (2), np/5 (5)	Severe
P. vannamei not exposed[e]	1.84	0	0	0	0	0	0	—	—	—	—	—	20 of 20	np/10 (0), 0/10 (5)	Negative

a. Shrimp all injected with 0.02 ml inoculum except *P. aztecus,* which was injected with 0.08 ml.
b. Source of *P. vannamei:* SPF from Kona, Hawaii.
c. Negative control specimens for this bioassay were injected with extract of noninfected shrimp.
d. np, not processed for histological examination.
e. Negative control specimens for this bioassay were injected with NaCl solution.

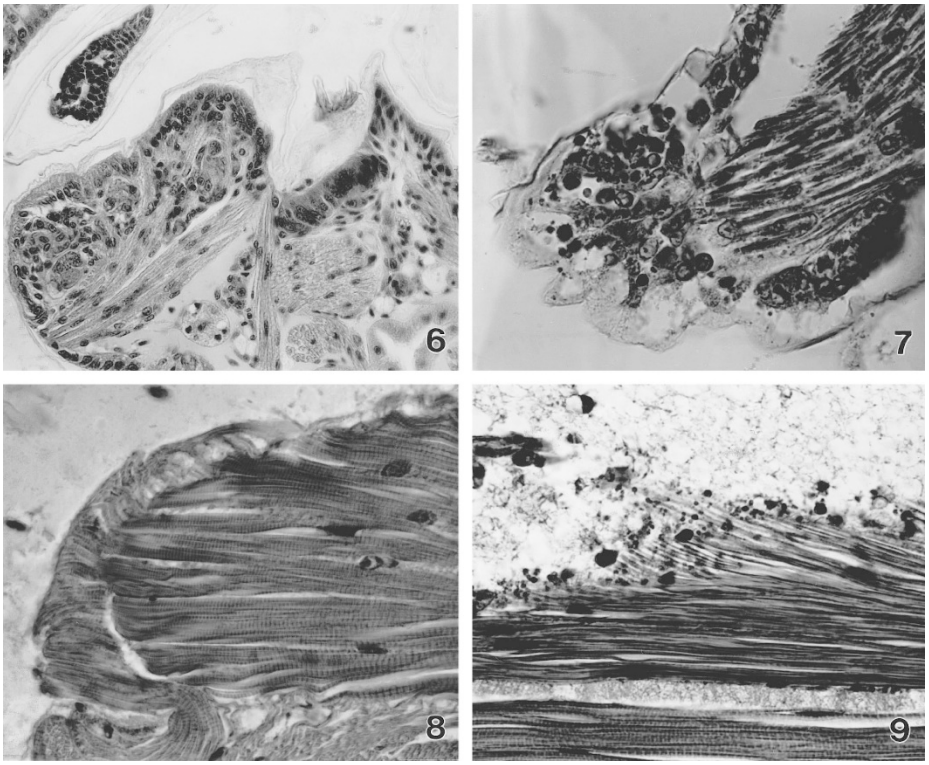
Table 7. Exposure of Wild Subadult Individuals of *P. setiferus* from Mississippi by Intramuscular Injection of a Filtrate of Taura Syndrome Virus, Using *P. vannamei* as Positive and Negative Controls

Group[a]	Average length (in mm)	Daily mortalities 0	1	2	3	4	5	6	7	8	9	10	11	Survivors of *N*	Remarks
P. setiferus exposed	79	0	0	2	3	5	1	0	0	0	2	—[b]	—	0 of 15	2 fixed Day 4
P. setiferus not exposed	79	0	0	0	0	0	4	0	0	0	0	0	0	3 of 8	Unexplained deaths on Day 5, 1 fixed Day 5
P. vannamei exposed[c]	83	0	0	5	3	0	2	0	1	1	0	0	0	3 of 15	3 fixed Day 3
P. vannamei not exposed[c]	83	0	0	0	0	0	0	0	0	0	0	0	0	7 of 7	

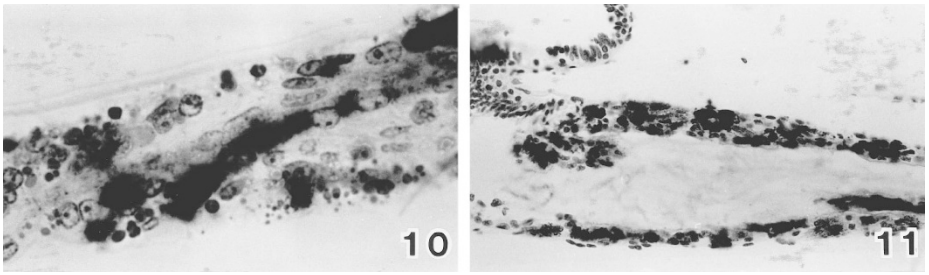
a. All shrimp injected with 0.02 ml filtrate.
b. All shrimp dead.
c. Source of *P. vannamei:* produced at GCRL from offspring of wild Guatemalan broodstock which had undergone quarantine at GCRL (PL 352, 359).



Figures 1–5. Hematoxylin and eosin sections of *Penaeus setiferus,* with some shrimp experimentally infected with Taura syndrome virus. 1. Gills from negative control shrimp. 2. Gills of shrimp demonstrating lesions of TSV. 3. Longitudinal section of antenna from negative control shrimp. 4. Longitudinal section of antenna from shrimp demonstrating lesions of TSV. 5. Frontal section of exopod of uropod from shrimp demonstrating lesions of TSV.



Figures 6–9. Hematoxylin and eosin sections of *P. setiferus*, with some shrimp experimentally infected with Taura syndrome virus. 6. Exopodite base of antenna from negative control shrimp. 7. Base of third maxilliped from shrimp demonstrating lesions of TSV. 8. Sagittal section of skeletal musculature of sixth abdominal segment and sinus from negative control shrimp. 9. Sagittal section of skeletal musculature of sixth abdominal segment and sinus from shrimp demonstrating lesions of TSV.



Figures 10–11. Histological sections of *P. setiferus* reacted with a digoxigenin-11–dUTP-labeled cDNA probe to Taura syndrome virus. The probe reacts with the virus-laden cytoplasm but not with the pyknotic and karyorrhectic nuclei of the necrotic cuticular epithelial cells and subcuticular connective tissue cells, results of and diagnostic features for the infection. 10. Pleopod showing "peppered" or "buckshot-riddled" appearance and positive dark probe reaction in the cytoplasm. 11. Hindgut epithelium showing strong reaction in cytoplasm.

Material of surviving exposed specimens of *P. setiferus* from studies reported in Tables 1 and 4 as well as that of *P. aztecus* and *P. duorarum* reported in Table 1 all produced mortalities from TSV when a supernate of their tissues was injected into one stock of bioassay *P. vannamei* but not when fed to another younger stock (Table 8). TSV infections were confirmed histologically with sections of some moribund shrimp injected with material from exposed *P. setiferus* and *P. vannamei*.

Table 8. Bioassay of TSV-Exposed Groups of Penaeid Shrimps Administered by Injection or Ingestion to TSV-Susceptible *P. vannamei* as Bioassay Animals

Group (source of material)	No. of days postexposure	Method of administration	*N*	Day of initial death	Survival on Day 6	Survival on Day 10	Confirmed histologically
TX-1 *P. setiferus*	19	Inject in juvenile	5	2	3	3	
TX-2 *P. setiferus*	14	Inject in juvenile	5	2	0	0	Yes
SC *P. setiferus*	20	Inject in juvenile	5	3	1	1	Yes
MS *P. setiferus*	20	Inject in juvenile	5	6	3	0	Yes
SC *P. aztecus*	79	Inject in juvenile	5	5	3	0	
SC *P. duorarum*	79	Inject in juvenile	5	5	2	0	Yes
Negative control-1[a]	—	Sham inject in juvenile	5	9	5	4	
Negative control-2[a]	—	Sham inject in juvenile	5	—	5	5	
TX-1 *P. setiferus*	19	Fed to young juvenile	15	10	13	13	
TX-2 *P. setiferus*	14	Fed to young juvenile	15	—	15	15	
SC *P. setiferus*	20	Fed to young juvenile	15	24	15	15	
MS *P. setiferus*	20	Fed to young juvenile	15	—	15	15	
SC *P. aztecus*	79	Fed to young juvenile	15	17	15	15	
SC *P. duorarum*	79	Fed to young juvenile	15	—	15	15	
Negative control-1[b]	—	Fed to young juvenile	15	—	15	15	
Negative control-2[b]	—	Fed to young juvenile	15	—	15	15	

Sources of *P. vannamei:*

a. produced at GCRL from high health status broodstock received from The Oceanic Institute, Hawaii (PL 153, 154) or
b. produced at Waddell Mariculture Center (WMC), South Carolina Department of Natural Resources, with 23 female SPF broodstock sent from Kona, Hawaii (PL 91).

Injection of *P. chinensis* with TSV resulted in a cumulative mortality of 70%, with no deaths occurring among the corresponding negative control shrimps. Histological analysis of those representative negative control and TSV-challenged *P. chinensis*, however, showed that a mild to moderate HPV infection was present in some of the shrimp. Mild TSV lesions, in comparison to the more severe lesions in exposed *P. vannamei*, were observed in two TSV-injected specimens that were collected in a moribund state.

Discussion

The outcome of this study is important because the knowledge of whether native shrimp can become infected or otherwise transmit TSV to other shrimp has an influence on assessing the health and potential health of both individuals and stocks of both native and cultured species. For example, local wild shrimp populations could become infected, become greatly reduced in number, and support transmission to adjacent stocks. Cultured shrimp could contract infections when water from farm supply canals or other sources containing contaminated shrimp or other material is pumped into ponds or other facilities. The source of the TSV and presumably other disease agents in ponds or natural bodies of water could be untreated shrimp waste from restaurants, seafood suppliers, processing plants, homes, or other ponds. Moreover, frozen or fresh infected shrimp could be used as bait or be deposited in landfills or other refuge sites, later to be transmitted to natural bodies of water by seagulls, other birds, water boatman, other insects, other animals, or other means.

The fact that *P. setiferus* can be infected experimentally with TSV means that infective material should be kept from sources that could contaminate that or other animal species. The fact that in some cases, specific exposed stocks of this or other species of shrimp do not readily succumb does not mean that there is no potential danger. An infected shrimp may or may not exhibit clinical signs of infection and may or may not die from a TSV infection, depending on the species and probably several other features involving the host, virus, and environmental conditions. The ability of *P. setiferus* and at least two other penaeid species to serve as carriers of the disease for at least 79 days strongly reflects the need for extreme care to keep infective material away from natural water bodies.

Even though infected, all individuals did not demonstrate characteristic histologic lesions of TSV. Infections cannot necessarily be confirmed histologically using bright-field H&E-sectioned material. The reason a lower percentage of specimens of *P. setiferus* exhibited lesions than of *P. vannamei* over a more restricted period probably indicates a difference in susceptibility. Still, if an entire shrimp was serially sectioned, rather than portions in representative planes, there would probably be some histological lesions diagnostic of infection. As dependable genomic probes or other biochemical techniques become more perfected, infections should probably be more obvious. At present, mortalities coupled with histological evidence, plus few or no mortalities in the nonexposed controls, constitute strong evidence of TSV-caused mortalities.

Results involving age of shrimp do not indicate a difference in susceptibility by age but are not conclusive. Our bioassays suggest that dose, which can be measured presently only in relative amounts, and a genetic difference in stocks contributes to some of the differences in mortality resulting from infection, regardless of whether the host is *P. setiferus*, *P. vannamei*, or some other species. For example, a Texas stock of *P. setiferus* used in our studies was susceptible to infection and mortality but less so than stocks from Mississippi and South Carolina. Genetic differences among those stocks probably have a strong influence on resulting pathogenicity from TSV. Erickson et al. (1996) reported that an experimental feeding of 15% biomass of TSV-infected *P. vannamei* over 4 days did not have an effect on juvenile specimens of *P. setiferus*, *P. aztecus*, and *P. duorarum* in outdoor tanks in Texas but

did kill over 90% of the *P. vannamei*. We infected specimens of *P. setiferus* from Texas, but they appeared less likely than *P. vannamei* and other stocks of *P. setiferus* to die from an infection. This example is not to imply that all wild shrimp from Texas or any other area are more or less resistant than wild shrimp from other specific areas.

We had good survival of our control shrimp, especially during the first 10 days of an experiment. There were a few cases involving mortalities that did not seem to be related to TSV. For example, the mortalities recorded for the *P. setiferus* control (13%) and treatment (7%) groups in Table 5 can best be attributed to cannibalism of recently molted shrimp. Water quality 2 days prior to mortalities of control wild *P. setiferus* on Day 5 in Table 7 appeared normal, but all died during the night.

The fact that *P. chinensis*, reared from Korean stock, can be experimentally infected with TSV illustrates how a virus from the western hemisphere could infect at least one commercially important shrimp from Southeast Asia, if given the opportunity. Experiments involving relationships involving co-infections with different viruses such as HPV should provide valuable information to biologists and shrimp farmers.

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