Summary of Taura syndrome virus (TSV) infection challenges

EXECUTIVE SUMMARY:

At the request of Biosecurity Australia, the University of Arizona challenged *Fenneropenaeus merguiensis* with the Thai isolate of Taura Syndrome Virus (TSV) and challenged *Penaeus monodon, Cherax quadricarinatus, Cherax tenuimanus* and *Macrobrachium rosenbergii* with the Thai and Belize isolates of TSV to determine whether or not these species were susceptible to TSV by *per os* (ingestion of infected prawn meat) or by injection challenge.

Results of TSV challenges on *Fenneropenaeus merguiensis* and *Penaeus monodon* indicate that these species can be infected with TSV but that the virus did not cause severe infections which would produce significant mortality. The *F. merguiensis* became infected with the Thai isolate of TSV by injection but did not become infected by *per os* challenge. The *P. monodon* did not become infected with the Thai isolate of TSV but some were infected with the Belize isolate of TSV by injection challenge only. Infection is defined for the purposes of this report as the presence of an actively replicating agent within the host and not its mere presence.

Results of the TSV challenges performed on *Cherax quadricarinatus, Cherax tenuimanus* and *Macrobrachium rosenbergii* indicate that these species can retain or sequester the virus but the virus does not form an active (replicative) infection. Therefore, none of these three species were infected with either the Thai isolate or Belize isolate of TSV.

SUMMARY OF RESULTS OF EACH CHALLENGE STUDY:

Each section below is intended to give a brief summary of the details and survival rates for each species. At the end of the report, tables are included to provide a brief overview of the survival and pathology results. Table 2 summarizes all the prawn challenge study results and Table 3 summarizes all the crayfish study results (including the *in situ* hybridization or ISH). Additionally, Table 4 is a complete summary of all of the ISH results for each study.

For the purposes of this summary, infection means the presence of an actively replicating agent within the host and not its mere presence. By the presence of an infectious agent in the host we mean detection of the agent in the host which may mean it was retained or sequestered after uptake by the host or by mechanical uptake from the gut or from the inoculum, or ingested tank debris, cannibalism, imbibition of tank water etc. Each species was not considered to be infected with the virus unless the virus was detected by histological methods or by ISH. A positive polymerase chain reaction (PCR) result indicated that the virus was present, but this method did not indicate whether the virus produced an active (replicating) infection. Hence, representative parallel samples from experimental groups with positive PCR test results were tested by ISH to determine the presence or absence of infection.

Banana Prawn (*Fenneropenaeus merguiensis*) UAZ Case 05-062

A total of 52 early juvenile *Fenneropenaeus merguiensis* were challenged with Thai isolate TSV by injection and *per os* to determine if *Fe. merguiensis* are susceptible to TSV.

At termination of the study, combined survival in the *per os* challenged group was 45% and combined survival in the injection group was 50%. Termination survival in the negative control tank was 60%.

Analysis of *Fe. merguiensis* samples taken post-challenge with TSV (Thai isolate) showed persistent infection by TSV of young juveniles of this species when challenged by direct injection of the virus, but significant disease was not a consequence of infection. *Per os* challenge was not as efficient as injection in inducing infection in experimental animals. A single positive animal was found by reverse transcriptase polymerase chain reaction (RT-PCR) on day 9 after *per os* challenge, but histopathology showed no signs of detectable infection (i.e. acute lesions or lymphoid organ spheroids which would have indicated the establishment of an acute or a persistent infection.

TSV was not detected by ISH in the animals challenged by the *per os* method. A low grade TSV infection was detected by ISH in one sample collected on day 9 of the injection portion of the challenge. Please refer to Table 2 for a summary of the pathology findings in the *Fe. merguiensis* study.

Marron Crayfish (*Cherax tenuimanus*) UAZ Case 05-162

A total of 270 juvenile *Cherax tenuimanus* were challenged with the Thailand and Belize isolates of TSV by injection and *per os* routes to determine if *C. tenuimanus* were susceptible to infection by either of these TSV strains.

At termination of the study, combined survival in the Thai TSV isolate *per os* challenged group was 48% and combined survival in the injection group was 50%. Combined survival in the *per os* challenged Belize isolate group was 52% and 58% in the injection challenged group. Termination survival in the negative control tank was 63%.

Histological and ISH findings collectively indicate that *C. tenuimanus* retains or sequesters TSV (whether challenged by injection or *per os*) leading to positive RT-PCR test results at least out to the termination (day 14) of the study, but that the virus does not form an active (replicative) infection. Please refer to Table 3 for a summary of the pathology findings.

Of interest was the presence of severe phycomycete (fungus) infections of the gills in several specimens of this species. This, along with the microsporidian infection also observed, may have contributed to the generally poor survival of this group.

Redclaw Crayfish (*Cherax quadricarinatus*) UAZ Case 05-205

A total of 270 juvenile *Cherax quadricarinatus* with were challenged individually with the Thai and Belize isolates of TSV to determine if *C. quadricarinatus* was susceptible to either of these TSV strains.

At termination of the study, combined survival in the Thai TSV *per os* challenged group was 85% and combined survival in the injection group was 85%. Combined survival in the orally challenged Belize TSV group was 89% and 90% in the injection challenged group. Termination survival in the negative control tank was 83%.

The histological and ISH findings indicate that this crayfish species retains or sequesters TSV (when directly challenged by injection with Belize TSV) leading to positive RT-PCR test results at least out to day 10 of the 14-day study, but that the virus does not form an active (replicative) infection. Please refer to Table 3 for a complete summary of all pathology findings.

An unrelated but interesting finding in this group was the presence in histological preparations from one specimen (specimen 05-205/A1) of a severe systemic infection by a rickettsia. The infection was presented as large basophilic cytoplasmic inclusion bodies in a variety of tissues, but primarily in the intertubular connective tissues of the hepatopancreas and in the gills That the agent contained in the inclusions was a rickettsia was confirmed by a tissue Gram stain and by ISH with a DNA probe to a conserved sequence of 16S rDNA from a rickettsia-like agent previously isolated from *P. monodon*.

Giant Tiger Prawn (*Penaeus monodon*) UAZ Case 05-306

A total of 135 juvenile *Penaeus monodon* were challenged with the Thai or Belize strains of TSV by injection and *per os* infection exposure to determine their susceptibility to either virus strain.

At termination of the study, combined survival in the Thai TSV isolate *per os* challenged group was 80% and combined survival in the injection group was 77%. Combined survival in both the *per os* challenged and injection challenged Belize TSV isolate groups was 80% and termination survival in the negative control tank was 53%.

PCR results indicated the presence of TSV in this stock of *P. monodon* (whether challenged by injection or *per os*), but the virus either did not produce infections of sufficient severity to be detectable by routine histology, or the timing of the sampling (and number of shrimp available for sampling) was such that acute infections were missed.

TSV was not detected in ISH assays performed on *P. monodon* from the injection or *per os* challenged Thailand isolate TSV challenge. TSV was also not detected in the *per os* challenged Belize isolate group, but was detected in one sample from the injection group challenged with the Belize isolate of TSV. Complete pathology results for this species can be found in Table 2.

Giant Freshwater Prawn (*Macrobrachium rosenbergii*) UAZ Case 05-344

A total of 270 juvenile *Macrobrachium rosenbergii* were challenged with the Thai and Belize isolates of TSV to determine if the species was susceptible to either or both virus strains.

At termination of the study, combined survival in the Thai TSV isolate *per os* challenged group was 63% and combined survival in the injection group was 58%. Combined survival in the Belize TSV isolate *per os* challenged group was 65% and the injection challenged group was 58% at termination. Termination survival in the negative control tank was 30%.

The results of the histological, ISH and RT-PCR assays indicate that this stock of *M. rosenbergii* was not infected with either the Belize or Thai isolates of TSV. A summary of all pathology results for this species can be found in Table 2.

TABLE 1. Summary of all Sources of Prawns and Crayfish Utilized in the Biosecurity Australia TSV Challenges.

Common Name **Scientific Name**	**Source** **Facility**	**Facility** **Location**
Black Tiger Prawn *Penaeus monodon*	Australian Prawn Farms	Queensland, Australia
Banana Prawn *Fenneropenaeus merguiensis*,	Bribie Island Aquaculture Research Centre	Queensland, Australia
Giant Freshwater Prawn *Macrobrachium rosenbergii*	Kentucky Aquaculture Research Center	Kentucky, USA
Redclaw Crayfish *Cherax quadricarinatus*	Ironbark Redclaw	Queensland, Australia
Marron Crayfish *Cherax tenuimanus*	CA & MA Nagle	Western Australia

TABLE 2. Summary of Results of all TSV Challenges on Penaeid Prawns for Biosecurity Australia.

Species and Life Stage	Route of Challenge	TSV Tissue Strain	Percent Survival			RT-PCR Results	ISH Results	Histology Results
			Challenge Animals	Negative Controls	Kona Positive Control			
F. merguiensis Early juvenile	*Per os*	Thailand	45%	60%	0%	Positive	Negative	TSV Not Detected
F. merguiensis Early juvenile	Injection	Thailand	45%	50%	0%	Positive	Positive	TSV Not Detected, but LOS lesions suggestive of chronic TSV detected
P. monodon Juvenile	*Per os*	Thailand	80%	53%	47%	Positive	Negative	TSV Not Detected *Vibrio*** infection
P. monodon Juvenile	Injection	Thailand	77%	53%	47%	Positive	Negative	TSV Not Detected *Vibrio* and *Zoothanium* infections
P. monodon Juvenile	*Per os*	Belize	80%	53%	40%	Positive	Negative	TSV Not Detected G1 HPV Infections, *Vibrio* infection
P. monodon Juvenile	Injection	Belize	80%	53%	40%	Positive	Positive	TSV Not Detected G1-G4 HPV Infection, *Vibrio* infection
M. rosenbergii Juvenile	*Per os*	Thailand	63%	30%	33%	Negative	NR*	TSV Not Detected Low grade *Vibrio* infection noted
M. rosenbergii Juvenile	Injection	Thailand	58%	30%	33%	Negative	NR	TSV Not Detected Low grade *Vibrio* infection noted
M. rosenbergii Juvenile	*Per os*	Belize	65%	30%	0%	Negative	Negative	TSV Not Detected Low grade *Vibrio* infection noted
M. rosenbergii Juvenile	Injection	Belize	58%	30%	0%	Positive	Negative	TSV Not Detected Low grade *Vibrio* infection noted

* NR - Samples not run ** '*Vibrio* infection' - bacteria observed were presumed to be a *Vibrio* species.
LOS - lymphoid organ spheroid HPV - hepatopancreatic parvovirus

TABLE 3. Summary of Results of all TSV Challenges on Crayfish for Biosecurity Australia.

Species and Life Stage	Route of Challenge	TSV Tissue Strain	Percent Survival			RT-PCR Results	ISH Results	Histology Results
			Challenge Animals	Negative Controls	Kona Positive Control			
Cherax quadricarinatus Juvenile	*Per os*	Thailand	85%	83%	23%	Negative	NR*	TSV Not Detected G1-2 bacterial infection
C. quadricarinatus Juvenile	Injection	Thailand	85%	83%	23%	Negative	NR	TSV Not Detected G1-2 bacterial infection
C. quadricarinatus Juvenile	*Per os*	Belize	89%	83%	0%	Negative	NR	TSV Not Detected G1 bacterial infection
C. quadricarinatus Juvenile	Injection	Belize	90%	83%	0%	Positive	Negative	TSV Not Detected Bacterial infection noted
Cherax tenuimanus Juvenile	*Per os*	Thailand	48%	63%	3%	Positive	Negative	TSV Not Detected G1-2 *Agmasoma* sp. (microsporidian) and protozoa infections noted
C. tenuimanus Juvenile	Injection	Thailand	50%	63%	3%	Positive	Negative	TSV Not Detected G1-2 Bacterial and *Lagenophrys*-like protozoan noted
C. tenuimanus Juvenile	*Per os*	Belize	52%	63%	3%	Positive	Negative	TSV Not Detected G2 *Leucothrix* sp. infection
C. tenuimanus Juvenile	Injection	Belize	58%	63%	3%	Positive	Negative	TSV Not Detected G2 *Zoothanium*, G2 *Leucothrix* sp and G2 *Episylis* sp. infections

* NR - Samples not run

TABLE 4. Summary of all *In Situ* Hybridization Assays from all Biosecurity Australia TSV Challenges.

ID Number	Species	Treatment	Reason Selected	ISH/TSV probe Result
05-049A/1	*L. vannamei*	Positive control	N/A	Positive probe reaction to acute phase lesions.
93-125B/5b	*L. vannamei*	SPF control	N/A	TSV not detected (no probe reaction to any tissue).
05-62 ⊂ Banana prawn (*Fenneropenaeus merguiensis*)				
05-62C/1-2 day 15	*Fenneropenaeus merguiensis*	*Per os* / Thai TSV	Day 9 RT-PCR +	2 of 2: TSV not detected.
05-62D/2-3 day 15	*Fe. merguiensis*	Injection / Thai TSV	Random check	2 of 2: TSV not detected.
05-62E/2-3 day 15	*Fe. merguiensis*	Injection / Thai TSV	Day 9 RT-PCR +	E/2: Low grade (G1) multifocal TSV positive lesions in the lymphoid organ. E/3: TSV not detected.
05-62F/1-2 day 15	*Fe. merguiensis*	Injection / Thai TSV	Day 9 RT-PCR +	2 of 2: TSV not detected.
05-162 ⊂ Marron crayfish (*Cherax tenuimanus*)				
05-162C/2 day 14	*Cherax tenuimanus*	*Per os* / Thai TSV	Day10 RT-PCR + & Suspect by H&E	TSV not detected
05-162D/1 day 14	*Cherax tenuimanus*	*Per os* / Thai TSV	Day 10 RT-PCR + & Suspect by H&E	TSV not detected
05-162E/4 day 14	*Cherax tenuimanus*	Injection / Thai TSV	Day10 RT-PCR + & Suspect by H&E	TSV not detected
05-162F/1	*Cherax tenuimanus*	Injection / Thai TSV	Day10 RT-PCR + &	TSV not detected

ID Number	Species	Treatment	Reason Selected	ISH/TSV probe Result
day 8			Suspect by H&E	
05-162H/3 day 14	*Cherax tenuimanus*	*Per os* / Belize TSV	Day10 RT-PCR + & Suspect by H&E	TSV not detected
05-162J/1 day 4	*Cherax tenuimanus*	*Per os* / Thai TSV	Suspect by H&E	TSV not detected
05-162K/2 day 12	*Cherax tenuimanus*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected
05-205 ⊂ Redclaw crayfish (*Cherax quadricarinatus*)				
05-205K/12 day 14	*C. quadricarinatus*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected
05-205K/13 day 14	*C. quadricarinatus*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected
05-205M/11 day 14	*C. quadricarinatus*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected
05-205M/13 day 14	*C. quadricarinatus*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected

05-306 ⊂ Giant tiger prawn (*Penaeus monodon*)				
05-306C/3-4 day 14	*Penaeus monodon*	*Per os* / Thai TSV	Day10 RT-PCR +	TSV not detected
05-306D/3-4 day 14	*Penaeus monodon*	*Per os* / Thai TSV	Day10 RT-PCR +	TSV not detected
05-306E/3-4 day 14	*Penaeus monodon*	Injection / Thai TSV	Day10 RT-PCR +	TSV not detected
05-306J/3-4 day 14	*Penaeus monodon*	*Per os* / Belize TSV	Day10 RT-PCR +	TSV not detected
05-306K/3-4 day 14	*Penaeus monodon*	Injection / Belize TSV	Day10 RT-PCR +	TSV not detected
05-306M/3-4 day 14	*Penaeus monodon*	Injection / Belize TSV	Day10 RT-PCR +	M/3:TSV not detected M/4: TSV positive cells in multifocal lesions in dorsal cuticular epithelium.
05-344 ⊂ Giant freshwater prawn (*Macrobrachium rosenbergii*)				
05-344J/11 day 14	*M. rosenbergii*	*Per os* / Belize TSV	Day 5 RT-PCR +; day 9 RT-PCR negative	TSV not detected
05-344K/6 day 6	*M. rosenbergii*	Injection / Belize TSV	Day 5 RT-PCR +; day 9 RT-PCR negative	TSV not detected
05-344M/12 day 14	*M. rosenbergii*	Injection / Belize TSV	Day 5 RT-PCR +; day 9 RT-PCR negative	TSV not detected