A Survey of Taura Syndrome Virus Intermediate Hosts on South Texas Shrimp Farms

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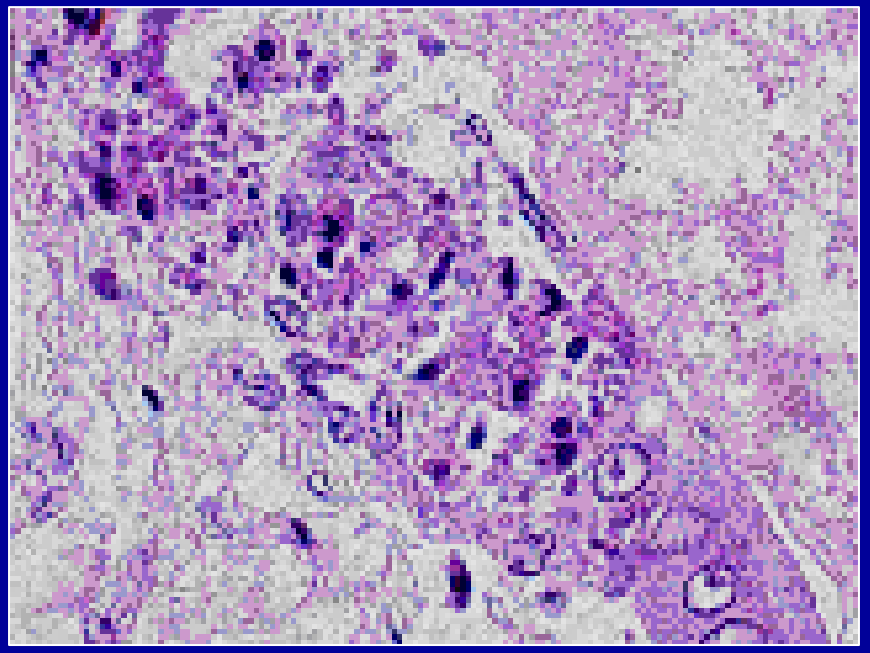
Introduction

- 1992 - Taura syndrome first encountered in Ecuador (Jimenez, 1992)
- Originally considered to be due to fungicide toxicity (Brock et al., 1995)
- Ultimately identified as a virus (Hasson et al., 1995)
- Outbreaks documented in farming regions of the Pacific (Hawaii), Central America, and the United States (Hasson et al., 1999)
- To date, three separate strains have been identified (Erickson et al., 2002; Robles-Sikisaka et al., 2002)

Introduction

Characteristics of TSV

- Originally classified as member of F. Picornaviridae (Bonami et al., 1997)
- Icosahedral morphology
- ss-RNA virus, genome 10205-bp in length
- Recently reclassified as Genus *Cripavirus* (F. Dicistroviridae) into the CrPV (Mari et al., 2002)



Acute TSV infection of shrimp epithelial cells (Ken Hasson, TVMDL)

Introduction

- Chronology of U.S. epizootics
  - 1994 (Hawaii, Florida)
  - 1995 (Texas, near 100% mortality)
  - 1996 (S. Carolina)
  - 2004 (50-60% mortality) (Gregg, pers. comm.)
    - Not all Texas farms affected, only those in the Rio Grande Valley

Modified from Lightner, 1995

Rationale for Study

- Mode of transmission of TSV into cultured shrimp populations is not well understood
- The only intermediate host described to date, and which is considered a transport vector of TSV (i.e., not infected by TSV) is the water boatman (*Trichocorixa reticulata*)
- Potential reservoirs could be highly variable, dependent upon environment and culture conditions, source of animals, etc.
- After 12 years, TSV remains an economic threat to farming operations in the U.S.

Research Hypothesis

Hypothesis: TSV vectors exist

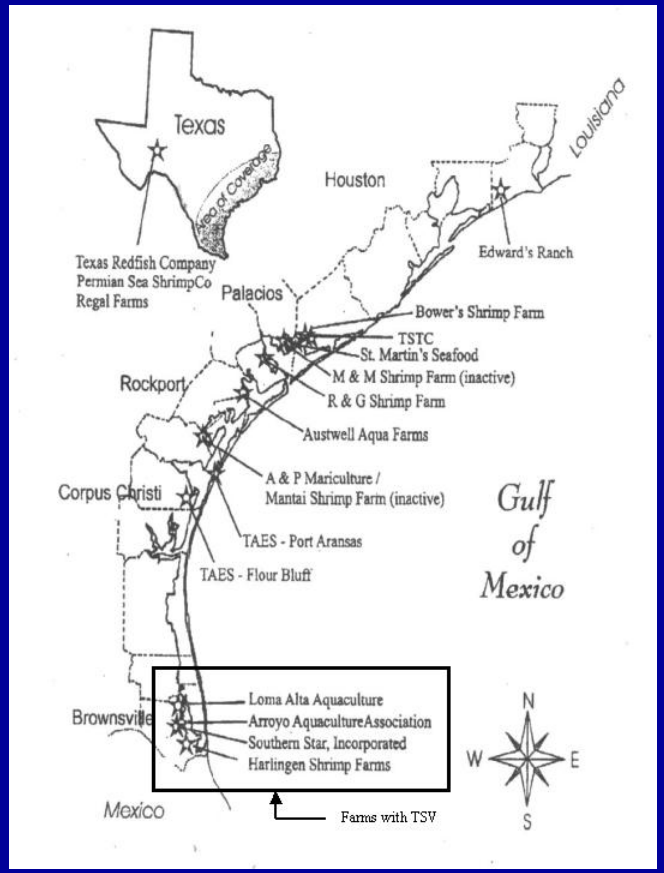
- Original virus could have come from infected reservoirs, but mutated to only affect shrimp
- Or: original virus has mutated to also allow infection of new hosts
- New hosts, in turn, can infect other shrimp
- **Bottom line**: not a static situation, no fixed number of intermediate hosts, situation will evolve

Study Objectives

1) Survey aquatic environment within quarantined shrimp farms for potential vectors;
2) Utilize RT-PCR to determine what micro/macro invertebrate samples contain TSV;
3) Evaluate monthly presence of TSV in “vector” samples and how this situation changes over time;
4) Compare findings between different farms having TSV.

Methods and Materials

- Sampled geographically separated shrimp farms in south Texas (Harlingen Shrimp Farms, Ltd.)
  - HSF-Bayview, Bayview, TX
  - Arroyo Aquaculture Association, Arroyo City, TX
  - Loma Alta Aquaculture, San Perlita, TX
- Global sampling regimen implemented (i.e., sample as many potential vectors as possible from each farm)



Methods and Materials

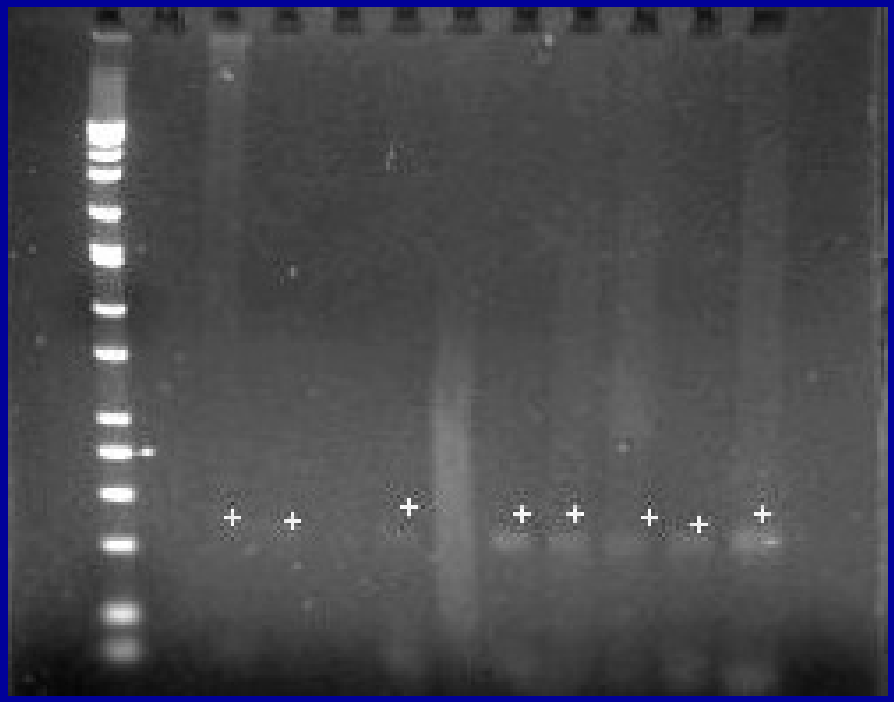
- Sampling for PCR analysis followed approved protocols (OIE, 2003) using 95% ethanol as a fixative
- Three ponds at each farm sampled bi-weekly throughout early summer and fall, 2004
- Sample classifications
  - shrimp (*Litopenaeus vannamei*)
  - zooplankton (various fractions, water column)
  - macroinvertebrates (various species; benthic, epibenthic, and pelagic)

Methods and Materials

- Shrimp obtained by cast net
- Zooplankton samples derived from singular 50 m plankton tows using various-mesh size nets (83, 153, 363 µm) at each sample site
- Macroinvertebrates obtained by dip net, sieves, benthic core or hand-grab
- Zooplankton samples concentrated by fraction, using gentle filtration onto Whatman No. 1 filters
- RT-PCR analysis of RNA conducted at TAMUCC according to Nunan (1998) using primers provided by UAZ Veterinary Microbiology Lab (Tucson, AZ)

Results, Plankton

- Overall mean prevalence of TSV in zooplankton for all three farms/ten ponds was 15%
- Many ponds surveyed (7/10) showed TSV in plankton tows on first sampling event
- Five out of ten initial plankton samples contained TSV



PCR gel of “planktonic” TSV

Results, Plankton

- Arroyo Aquaculture Assn. ponds had lower incidence of TSV associated with plankton samples (6.67%) vs. other farms (13.33, 22.22%)
- 30% of the Bayview plankton samples containing TSV consisted primarily of small *Palaemonetes* sp.
- Pond 21 (Arroyo Aquaculture Assn.) had TSV associated only with plankton (not with shrimp or macroinverts)
- Presence of plankton (and TSV in plankton samples) at times appeared cyclical (2-4 wk)

Results, Macroinvertebrates

- Overall mean prevalence in macroinvert's was relatively similar to that of plankton samples (13 vs. 15%)
- Prevalence relatively similar for all farms
- However: presence, availability of macroinvert's was highly variable, farm-to-farm
  - HSF-Bayview: barnacles (O. Cirripedia) and water boatmen (F. Corixidae)
  - Loma Alta: *Paleomonetes* sp. and water boatmen
  - Arroyo Aquaculture Assn.: primarily mussels (*Mytilus edulis*)

PCR gel showing TSV-positive mussels

Macroinvert Prevalence[1]

Farm Pond	Bayview			Loma Alta				Arroyo Aquaculture Association					
	G7	N5	N2	11	3	10	5	6	20	21	60	77	FC[2]
Amphipods	0	0	0				0		0				
Barnacles	0	20	0						0				
Mud crabs			0										0
Mussles									100	0	100	0	0
Niads				0		0					0		0
Palaeomonetes			0	50	0	0		0					
Polychaetes								0					
Water beetles					0								
Water Boatmen		50	0	0	100	0		0	0			0	

[1]Percent prevalence of TSV in samples. [2]Feeder canal.

Results, all farms[1]

Category	Bayview (%) ←	Arroyo Aquaculture (%)	Loma Alta (%) →
Shrimp	30.77	25.00	29.17
Plankton, total	22.22	6.67	13.33
83 µm	26.67	0.00	16.67
153 µm	13.33	6.67	8.33
363 µm	26.67	13.33	25.00
Macroinvertebrates	12.5	10.00	15.38

[1]values represent mean prevalence (%) of TSV in samples

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Other Issues

- Temporal aspect of samples
  - Loma Alta
  - Arroyo
  - Bayview

- Slight apparent correlation between prevalence of TSV in shrimp and plankton samples
- TSV in vector samples disappeared toward end of study once shrimp were gone

Discussion

- In general, association of potential vectors and TSV was relatively similar by vector group (plankton vs. macroinvert's)
- Appears to be some potential for vectors to transmit TSV to shrimp in that TSV was found in some vector samples prior to shrimp samples
- However, vectors could be simply concentrating TSV from shrimp (i.e., TSV present in shrimp, but at undetectable level)
- **Question**: is TSV in vectors purely a mechanical issue or did they actively infect shrimp? Cannot say from this study.

Discussion

- Presence and absence of TSV in plankton samples could represent cyclical nature of life cycle of planktonic species
- Hatching of *Palaemonetes* sp. larvae has been shown to follow a 2-week incubation/brooding cycle in the natural environment (Lund et al., 2000)
- Decline in presence of TSV in potential vectors upon harvest of shrimp suggests lack of persistence of TSV in environment
- Are vectors infected by extra-farm agents (e.g., other shrimp species) and then do they enter the ponds and infect pond shrimp? Many scenarios are possible, but none confirmed.

Discussion

- “Vectors” could serve as advanced warning of presence of TSV
- Future work should be comprehensive in evaluating source water biota prior to filling ponds, more frequent sampling with confirmation of active infection via histology and *in-situ* hybridization
- This is the plan for subsequent research funded by USDA-APHIS for 2005, contingent upon presence of TSV this coming season.

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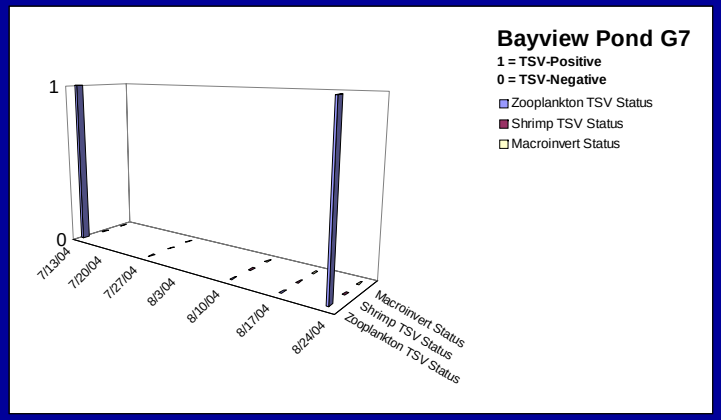
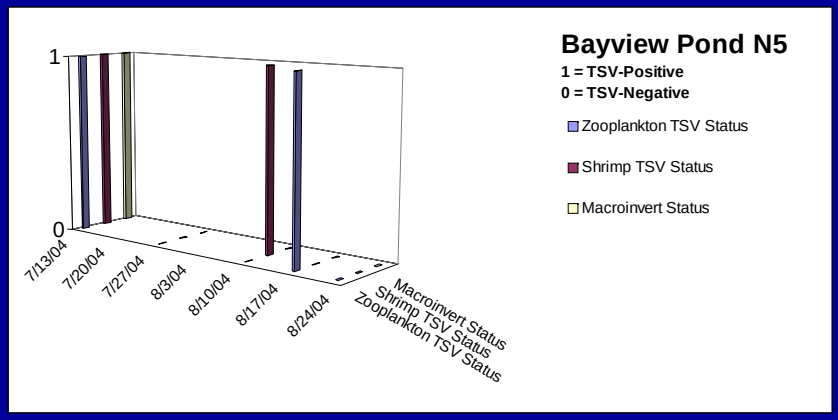
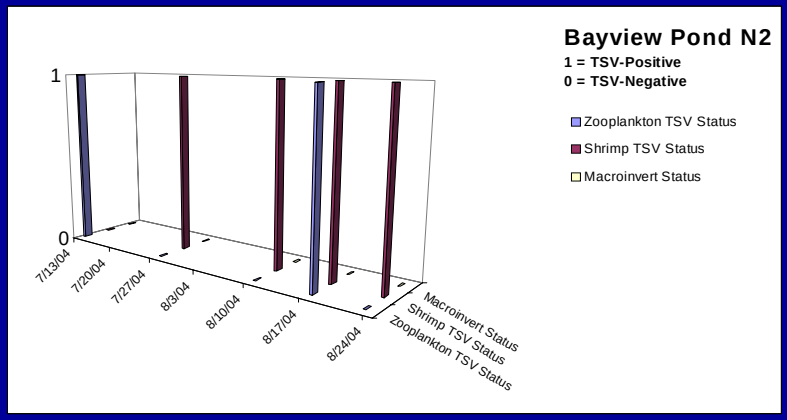
Bayview Data Charts







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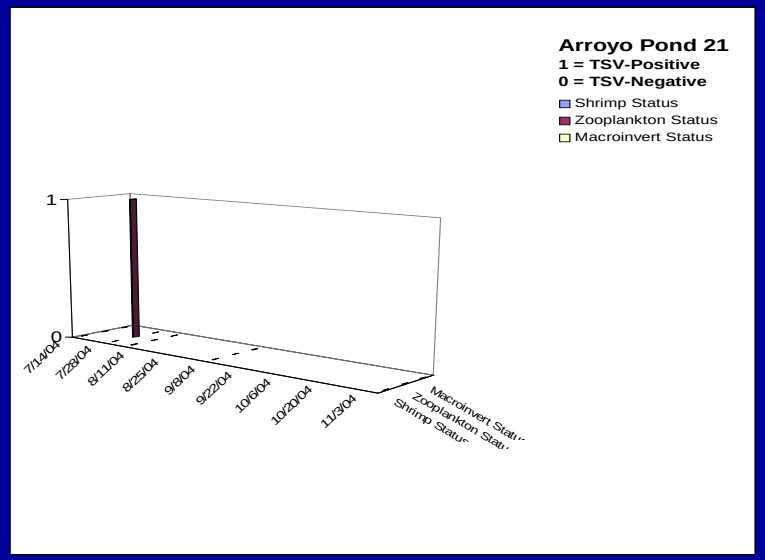
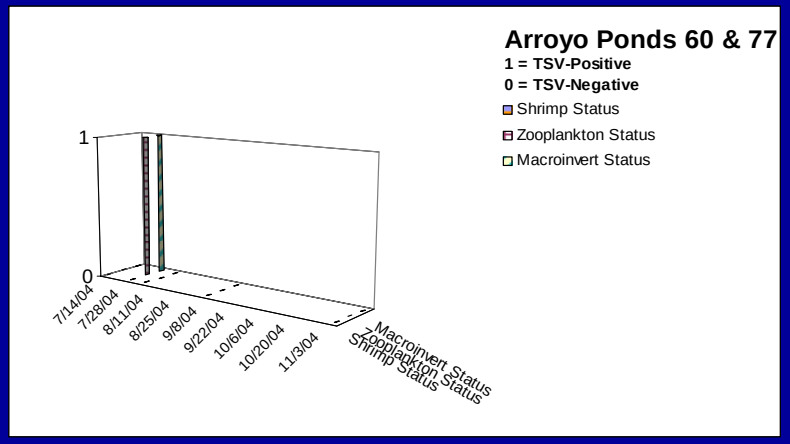
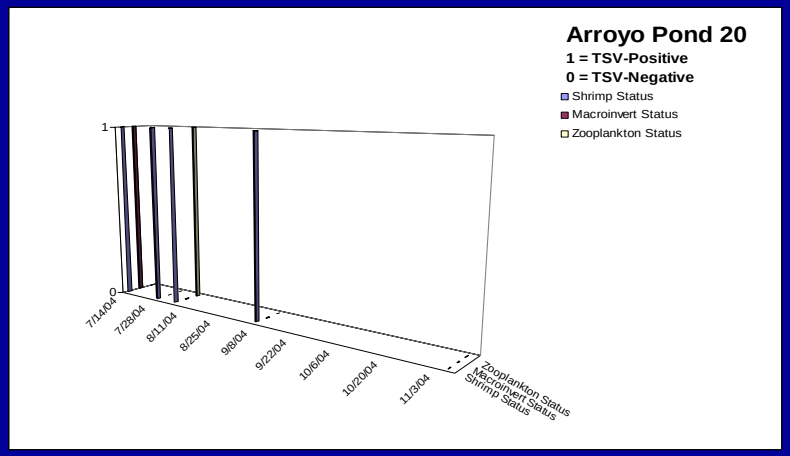
Arroyo Data Charts







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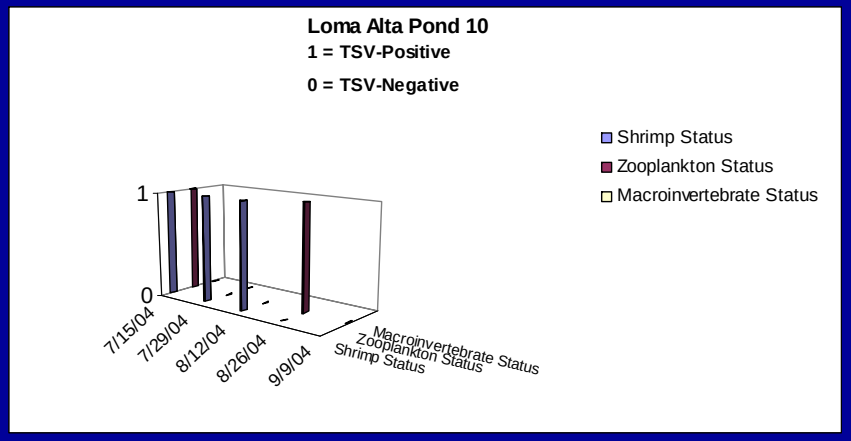
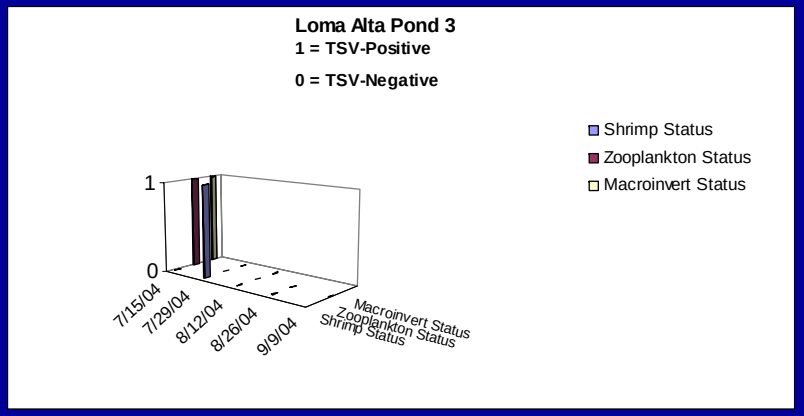
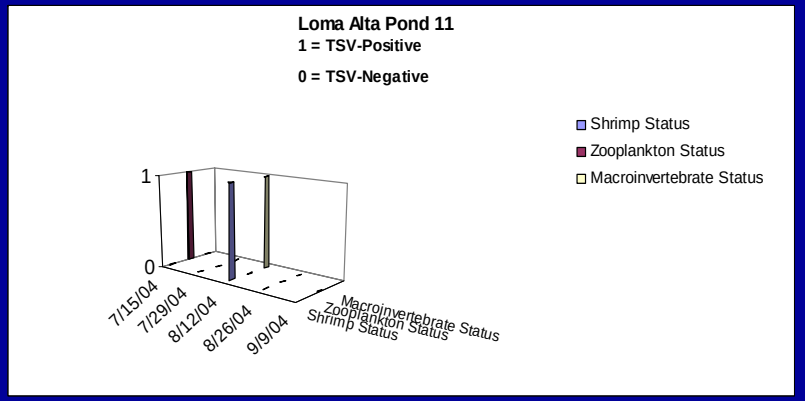
Loma Alta Data Charts







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