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AQUACULTURE PATHOLOGY
Tucson, Arizona

TO: [redacted]
Department of Agriculture, Water and the Environment (Canberra)

FROM: [redacted]
University of Arizona

DATE: 3 August 2022

RE: Final Report for UAZ Case 22-113, examination of infectivity of WSSV and YHV1 tissue when cooked at temperatures ranging from 60°C to 95°C

PURPOSE:

At the request of [redacted] of the Australian Department of Agriculture, Water, and the Environment, the University of Arizona (UAZ) fed specific pathogen free (SPF) *Penaeus vannamei* tissue which was previously infected with white spot syndrome virus (WSSV) or yellow head virus genotype 1 (YHV1). Infectious tissues were generated, frozen at -20ºC, and cooked at temperatures ranging from 60°C to 95°C prior to feeding to SPF *P. vannamei*. The purpose of this study was to observe whether cooked tissues would produce an infection in healthy *P. vannamei*.

AQUARIA AND ANIMALS:

There were two stages to each viral challenge. The first stage was the production of infectious tissue for both WSSV and YHV1. Two 1000 L tanks were stocked with 40 *P. vannamei* (average weight 3.5 g) per tank and fed tissue infected with each respective virus (see CHALLENGE METHODS). In stage 2, a total of 540 SPF *P. vannamei* (average weight 3.5 g) were stocked into twenty-eight 90 L tanks and used as challenge groups. See Table 1 for definitions of all tanks used in this study. Due to a shortage of available *P. vannamei*, the negative control tanks for each virus were stocked with 15 *P. vannamei* per tank while all challenge tanks were stocked with 20 per tank.

The *Penaeus vannamei* used in this study were obtained from a commercial producer and reared at UAZ. All tanks were outfitted with pre-acclimated biological filters, aeration and covered with plastic sheeting to contain aerosols and prevent escape. All tanks were backflushed and had water quality measurements recorded weekly. The salinity in each tank ranged from 29.8-31.4 ppt and temperatures were maintained at 28.0ºC (±0.5ºC). The average ammonia value for all tanks was 0.3 ppm and the average nitrite value was 0.0 ppm.

CHALLENGE METHODS:

Stage 1: Tissue Generation

The WSSV and YHV1 tissue generations were conducted via oral exposure by feeding infected tissues to SPF *P. vannamei*. Two 1000 L fiberglass tanks were stocked with 28 SPF *P. vannamei* (avg weight 3.5 g) into each tank. One tank was fed known WSSV-infected tissue from UAZ Case# 19-859 at 10% body weight for a single feeding on day 0 of the study. The second tank was fed known YHV1-infected tissue from UAZ Case #22-061 at 10% body weight for a single feeding. Mortalities were first noted 2 days post-infection in the WSSV tissue generation tank and 4 days post-infection in the YHV1 tissue generation tank. See Table 2 stocking details and timeline summary of the tissue generation tanks.

All mortalities observed in the tissue generation tanks prior to termination were frozen at -20°C. When the number of surviving *P. vannamei* fell to approximately 50% of the original stocking number, all *P. vannamei* were removed and the tank was terminated. Two *P. vannamei* displaying clinical symptoms from each tissue generation tank were preserved in Davidson's AFA for H&E histology, two samples were collected for PCR analysis, and the remaining survivors were frozen at -20ºC. The WSSV tissue generation tank was terminated on day 3 with 39.3% survival and the YHV1 tissue generation tank was terminated on day 6 with 53.6% survival. All remaining *P. vannamei* were sealed in plastic bags and frozen at -20ºC until the tissue was prepared for cooking in Stage 2.

Samples collected during the Stage 1 tissue generation were analyzed by both histology and qPCR. The WSSV and YHV1 tissues generated in Stage 1 were confirmed to be positive for each respective pathogen. See Table 3 for histology and qPCR results of the tissue generation tanks.

Stage 2: Cooking and Tissue Feeding

Prior to the start of Stage 2, twenty-eight 90 L aquaria were stocked with 15-20 SPF *P. vannamei* (avg weight 3.5 g) per tank (see Table 1). The WSSV and YHV1 tanks were each divided into 7 groups with 2 replicates per group. The first group for each virus was not fed infected tissue and served as negative control tanks.

Both WSSV and YHV1 had positive control groups which were fed uncooked, infected tissues at a rate of 10% of biomass for a single feeding. The remaining challenge tanks were fed tissue after the designated temperature was achieved for each virus.

Prior to cooking, all mortalities and survivors harvested in Stage 1 tissue generations were combined to form one tissue pool for each virus. Three *P. vannamei* were randomly removed from the tissue pool and two samples of gills/pleopods were collected for real-time and conventional PCR. Tissue from three infected *P. vannamei* was then minced and fed to each respective positive control tank at a rate of 10% of biomass for a single feeding. See Table 4 for a summary of the timeline for all stages in this study.

Twelve liters of tap water were added to a cooking pot and placed on a hot plate set to 100°C. There were five groups of required cooking temperatures: 60°C, 70°C, 75°C, 85°C, and 95°C. For each cooking temperature, three temperature probes (Soraken Wireless Thermometer, model 7539893357) were placed into the first tail segment of the frozen *P. vannamei* and the core temperatures were measured. The thermometers were placed in the center of the tails to record the core temperature of the tail segment. After the water achieved boiling temperature, the three frozen shrimp were then simultaneously placed into the cooking pot. As the *P. vannamei* cooked, the probes in the tail muscle recorded the internal temperature of the tissue (see Table 9).

As the required temperature of each *P. vannamei* sample was achieved, the cooked carcass was removed from the water and immediately cooled in an ice water bath. After cooling, samples of gills/pleopods from two *P. vannamei* from each temperature group were preserved in 95% ethanol for detection by conventional and real-time PCR. The remaining tissue was minced and fed to the respective challenge tank temperature group at a rate of 10% of body weight for a single feeding.

Tissue from each virus was prepared in this manner for each temperature group. WSSV cooking was conducted on day 4 of the study and YHV1 cooking was conducted on day 7.

Stage 3: Observation and Termination

After feeding the cooked tissues to the challenge tanks, all tanks were observed once per day until termination of each viral group. WSSV was terminated 14 days after infection and YHV1 was terminated 21 days after infection. All mortalities and moribund noted during this time were recorded and frozen at -80°C.

Up to two survivors per tank had tissue samples collected for real-time PCR and were then preserved in Davidson's AFA for examination by histology.

RESULTS:

Prior to the study, two SPF *P. vannamei* were preserved in Davidson's AFA to document the health status of the population used in this study. The shrimp were determined to be free of both WSSV and YHV1.

WSSV Stage 1 Tissue Generation:

The tissue produced in the WSSV tissue generation tank was determined to be positive by both PCR and histology. Analysis by real-time PCR of two moribund samples collected showed the tissue to have Ct values of 13.23 and 12.91 for a strong positive result and conventional PCR confirmation of the positive infection. Histological analysis of two *P. vannamei* collected during the tissue generation displayed Grade 4 lesions for WSSV infection (Table 3).

YHV1 Stage 1 Tissue Generation:

The tissue produced in the YHV1 tissue generation tank was determined to be positive by both

PCR and histology. Analysis by real-time PCR showed the two samples to display Ct values of 15.00 and 14.92, indicating a very high viral load and a confirmation of positive infection by conventional PCR. Histology of these samples displayed Grade 3-4 lesions for YHV1 infection (see Table 3).

WSSV Stage 2 Cooked Tissue Challenge:

I. Negative Control Group

The negative control tanks of the WSSV Stage 2 were stocked with fifteen shrimp per tank. No mortalities were noted in either tank until day 12. Due to external personnel conducting a repair in the room, the tank was accidentally damaged and experienced 100% mortality. The *P vannamei* in tank 1 were decomposed when the mortality was recorded but one sample was able to be preserved for histology. This sample was negative for WSSV. Mortalities were not noted in the second negative control tank for the duration of the study.

Due to the accidental mortality in tank 1, the overall survival for this group was only 33.3%. The second control tank had 10 of 15 shrimp survive at termination of the study for a total survival of 66.7%. This survival was lower than average and was most likely due to cannibalism, as the tank appeared active and healthy for the duration of the study.

Histology and PCR results of samples collected from tank 2 at termination were negative for WSSV. See figure 1 for a summary of the WSSV negative control group.

Fig. 1 *Summary of PCR and histology analyses performed on moribund samples collected from the WSSV positive control tanks. The Ct value was collected from real-time PCR. Histology grades are summarized in Appendix 1.*

Tank	Sample Type	Sample Day	Ct Value	Histology Grade	PCR Result	Histology Result
1	Moribund	12	ND*	ND	NEGATIVE	NEGATIVE
2	Healthy	17	ND	ND	NEGATIVE	NEGATIVE
2	Healthy	17	ND	ND	NEGATIVE	NEGATIVE

*ND = Not Detected

II. WSSV Positive Control Group

The WSSV positive control tanks exhibited clinical signs and mortality commonly associated with white spot syndrome infection. The first mortalities were noted in both tanks three days after feeding WSSV tissue. Tank 13 was terminated four days later and tank 14 was terminated only two days after the first mortalities were noted. Both tanks had 0% survival.

Both PCR and histology analysis of moribund samples confirmed WSSV infection in each tank in

Figure 2 below.

Fig. 2 *Summary of PCR and histology analyses performed on moribund samples collected from the WSSV positive control tanks. The Ct value was collected from real-time PCR. Histology grades are summarized in Appendix 1.*

Tank	Sample Type	Sample Day	Ct Value	Histology Grade	PCR Result	Histology Result
13	Moribund	10	13.23	G4	POSITIVE	POSITIVE
14	Moribund	10	12.91	G4	POSITIVE	POSITIVE

III. WSSV Cooked Tissue Groups

There were only 3 mortalities noted in the cooked tissue tanks during the challenge period. Two were noted on the day of infection, prior to feeding cooked tissues, in tanks 3 and 8 (one per tank). The final observed mortality was in tank 5 on day 11 (8 days after tissue feeding). The tanks ranged in survival from 60%-100% and all samples collected at termination of the study were negative by both PCR and histology for the presence of WSSV. See Table 7 for survival data of all groups.

YHV1 Stage 2 Cooked Tissue Challenge:

I. Negative Control Group

The negative control tanks of the YHV1 Stage 2 were stocked with fifteen shrimp per tank. Each tank had one recorded mortality, and both were observed on day 8. No additional mortalities were observed for the duration of the study. Each tank had 13 of 15 survivors at termination for an induvial and group survival rate of 86.7%

Histology and PCR results of samples collected form tank 2 at termination were negative for YHV1. See figure 1 for a summary of the YHV1 negative control group.

Fig. 3 *Summary of PCR and histology analyses performed on moribund samples collected from the YHV1 positive control tanks. The Ct value was collected from real-time PCR. Histology grades are summarized in Appendix 1.*

Tank	Sample Type	Sample Day	Ct Value	Histology Grade	PCR Result	Histology Result
1	Healthy	28	ND	ND	NEGATIVE	NEGATIVE
2	Healthy	28	ND	ND	NEGATIVE	NEGATIVE

II. *YHV1 Positive Control Group*

The YHV1 positive control tanks exhibited clinical signs and mortality commonly associated with yellow head virus genotype 1 infection. The first morality noted in tank 27 was recorded on day 15. This tank continued to exhibit reduced appetite, reduced activity levels, and mortality until it was terminated on day 21.

The first mortality was noted in tank 28 five days after feeding YHV1 tissue (day 12 overall). This tank was terminated on day 18 with no surviving *P. vannamei* remaining. UAZ was unable to obtain a suitable sample for histology in this tank. Tissue from dead *P. vannamei* was preserved for PCR analysis on day 18. PCR results were shown to be positive for YHV1, and this tissue displayed a strong infection (Ct value = 12.91) for YHV1 by qPCR.

The overall survival for the group was 0%. Both PCR and histology analysis of moribund samples confirmed YHV1 infection in each tank in Figure 2 below.

Fig. 4 *Summary of PCR and histology analyses performed on moribund samples collected from the YHV1 positive control tanks. The Ct value was collected from real-time PCR. Histology grades are summarized in Appendix 1.*

Tank	Sample Type	Sample Day	Ct Value	Histology Grade	PCR Result	Histology Result
27	Moribund	15	13.23	G3	POSITIVE	POSITIVE
28	Moribund	18	12.91	none	POSITIVE	none

III. *YHV1 Cooked Tissue Groups*

There were only two mortalities observed across all cooked tissue tanks during the challenge period. The tanks ranged in survival from 70%-100% and all samples collected at termination of the study were negative by both PCR and histology for the presence of YHV1. See Table 7 for survival data of all tanks by group in this study.

SUMMARY:

WSSV and YHV1 infections were successfully replicated in the tissue generation tanks. The tissues harvested from these tanks were also able to reliably produce an infection in each respective positive control tank. These same WSSV and YHV1 tissues, when cooked between 60ºC and 95°C, did not produce an infection by either virus in any of the challenge tanks. See overall survival summary for all tanks in Table 7.

PATHOLOGY:

Histological examination of the samples from the challenge was completed by [redacted] and Table 6 and Appendix 1 summarizes the histology findings from this case.

Sincerely,

[redacted]

Reviewed and Approved By,

[redacted]

Table 1. Definition of all tanks used in the Australian WSSV and YHV1 cooking study (UAZ Case# 22-113).

Tank	No Stocked	Avg Weight	Pathogen	Tank Designation	Tank Size	Cooking Temperature
B1	28	3.5 g	WSSV	WSSV Tissue Gen	1000 L	N/A
D1	28	3.5 g	YHV1	YHV1 Tissue Gen	1000 L	N/A
1	15	3.5 g	none	Neg Control	90 L	N/A
2	15	3.5 g	none	Neg Control	90 L	N/A
3	20	3.5 g	WSSV	Challenge	90 L	95ºC
4	20	3.5 g	WSSV	Challenge	90 L	95ºC
5	20	3.5 g	WSSV	Challenge	90 L	85ºC
6	20	3.5 g	WSSV	Challenge	90 L	85ºC
7	20	3.5 g	WSSV	Challenge	90 L	75ºC
8	20	3.5 g	WSSV	Challenge	90 L	75ºC
9	20	3.5 g	WSSV	Challenge	90 L	70ºC
10	20	3.5 g	WSSV	Challenge	90 L	70ºC
11	20	3.5 g	WSSV	Challenge	90 L	60ºC
12	20	3.5 g	WSSV	Challenge	90 L	60ºC
13	20	3.5 g	WSSV	Pos Control	90 L	Thawed
14	20	3.5 g	WSSV	Pos Control	90 L	Thawed
15	15	3.5 g	none	Neg Control	90 L	N/A
16	15	3.5 g	none	Neg Control	90 L	N/A
17	20	3.5 g	YHV1	Challenge	90 L	95ºC
18	20	3.5 g	YHV1	Challenge	90 L	95ºC
19	20	3.5 g	YHV1	Challenge	90 L	85ºC
20	20	3.5 g	YHV1	Challenge	90 L	85ºC
21	20	3.5 g	YHV1	Challenge	90 L	75ºC
22	20	3.5 g	YHV1	Challenge	90 L	75ºC
23	20	3.5 g	YHV1	Challenge	90 L	70ºC
24	20	3.5 g	YHV1	Challenge	90 L	70ºC
25	20	3.5 g	YHV1	Challenge	90 L	60ºC
26	20	3.5 g	YHV1	Challenge	90 L	60ºC
27	20	3.5 g	YHV1	Pos Control	90 L	Thawed
28	20	3.5 g	YHV1	Pos Control	90 L	Thawed

Table 2. Stocking details and mortality timeline of generation of WSSV and YHV1 tissue described in Stage 1 of each viral study.

Virus	Tank Size	No Stocked	Avg Weight	Tissue Fed	Mortality First Noted	Day Terminated	Survivors at Termination
WSSV	1000L	28	3.5 g	10% Once	Day 2	Day 3	11
YHV1	1000L	28	3.5 g	10% Once	Day 4	Day 6	15

Table 3. Histology and PCR summary of *Penaeus vannamei* collected during tissue generation of WSSV and YHV1 in Stage 1. These samples were collected after each respective tank displayed approximately 50% mortality.

Tank	Virus	Sample ID	Day Collected	Histology Grade	qPCR Ct Value	PCR Result
B1	WSSV	A3	3	G4	13.23	WSSV Positive
B1	WSSV	A4	3	G4	12.91	WSSV Positive
D1	YHV1	B1	6	G3	15.00	YHV1 Positive
D1	YHV1	B2	6	G4	14.92	YHV1 Positive

Table 4. Start and termination timeline for Australian Dept of Agriculture, Water, and Environment WSSV and YHV1 cooked tissue study (UAZ Case# 22-113).

	Day							
	0	3	4	6	7	8	18	28
WSSV Tissue Gen	Start	Terminate						
YHV1 Tissue Gen	Start			Terminate				
WSSV Cooking Chall			Start				Terminate	
WSSV Pos Control			Start			Terminate		
YHV1 Cooking Chall					Start			Terminate
YHV1 Pos Control					Start			Terminate

Table 5. PCR results of tissue samples of WSSV and YHV1 analyzed prior to cooking at respective temperatures (UAZ Case# 22-113).

Sample Name	Virus	Species	Result	Ct Value	Copies/ng of extracted DNA
Day0-60^{0}C	WSSV	*P. vannamei*	POSITIVE	14.01	2.21E+07
Day0-70^{0}C	WSSV	*P. vannamei*	POSITIVE	12.68	5.34E+07
Day0-75^{0}C	WSSV	*P. vannamei*	POSITIVE	13.41	3.30E+07
Day0-85^{0}C	WSSV	*P. vannamei*	POSITIVE	13.66	2.79E+07
Day0-95^{0}C	WSSV	*P. vannamei*	POSITIVE	12.75	5.10E+07
Day0-60^{0}C	WSSV	*P. vannamei*	POSITIVE	13.23	2.21E+07
WSSV Pos Control	WSSV	*P. vannamei*	POSITIVE	13.91	2.36E+07
Day0-60^{0}C	YHV1	*P. vannamei*	POSITIVE	26.30	6.13E+03
Day0-70^{0}C	YHV1	*P. vannamei*	POSITIVE	16.03	1.01E+06
Day0-75^{0}C	YHV1	*P. vannamei*	POSITIVE	23.10	3.01E+04
Day0-85^{0}C	YHV1	*P. vannamei*	POSITIVE	24.23	3.01E+04
Day0-95^{0}C	YHV1	*P. vannamei*	POSITIVE	22.38	4.29E+04
YHV 1 Pos Control	YHV1	*P. vannamei*	POSITIVE	25.29	1.01E+04

Table 6. PCR and Histology results of samples collected after cooking and feeding of tissue in Australian Dept of Agriculture, Water, and Environment WSSV and YHV1 cooked tissue study (UAZ Case# 22-113).

Tank	Pathogen	Sample Type	Ct Value	PCR Result	Histology Grade	Histology Result
1	Neg Control	Termination	ND	NEGATIVE	ND	NEGATIVE
2	Neg Control	Termination	ND	NEGATIVE	ND	NEGATIVE
3	WSSV 60 C	Termination	ND	NEGATIVE	ND	NEGATIVE
4	WSSV 60 C	Termination	ND	NEGATIVE	ND	NEGATIVE
5	WSSV 70 C	Termination	ND	NEGATIVE	ND	NEGATIVE
6	WSSV 70 C	Termination	ND	NEGATIVE	ND	NEGATIVE
7	WSSV 75 C	Termination	ND	NEGATIVE	ND	NEGATIVE
8	WSSV 75 C	Termination	ND	NEGATIVE	ND	NEGATIVE
9	WSSV 85 C	Termination	ND	NEGATIVE	ND	NEGATIVE
10	WSSV 85 C	Termination	ND	NEGATIVE	ND	NEGATIVE
11	WSSV 95 C	Termination	ND	NEGATIVE	ND	NEGATIVE
12	WSSV 95 C	Termination	ND	NEGATIVE	ND	NEGATIVE
13	WSSV Pos Control	Moribund	13.23	POSITIVE	Grade 4	POSITIVE
14	WSSV Pos Control	Moribund	12.91	POSITIVE	Grade 4	POSITIVE
15	Neg Control	Termination	ND	NEGATIVE	ND	NEGATIVE
16	Neg Control	Termination	ND	NEGATIVE	ND	NEGATIVE
17	YHV1 60 C	Termination	ND	NEGATIVE	ND	NEGATIVE
18	YHV1 60 C	Termination	ND	NEGATIVE	ND	NEGATIVE
19	YHV1 70 C	Termination	ND	NEGATIVE	ND	NEGATIVE
20	YHV1 70 C	Termination	ND	NEGATIVE	ND	NEGATIVE
21	YHV1 75 C	Termination	ND	NEGATIVE	ND	NEGATIVE
22	YHV1 75 C	Termination	ND	NEGATIVE	ND	NEGATIVE
23	YHV1 85 C	Termination	ND	NEGATIVE	ND	NEGATIVE
24	YHV1 85 C	Termination	ND	NEGATIVE	ND	NEGATIVE
25	YHV1 95 C	Termination	ND	NEGATIVE	ND	NEGATIVE
26	YHV1 95 C	Termination	ND	NEGATIVE	ND	NEGATIVE
27	YHV1 Pos Control	Moribund	21.65	POSITIVE	Grade 3	POSITIVE
28	YHV1 Pos Control	Fresh Mort	18.48	POSITIVE	No Sample	N/A

Table 7. Mortality summary for all tanks after feeding of cooked tissues for both WSSV and YHV1 in Australian Dept of Agriculture, Water, and Environment cooked tissue study (UAZ Case# 22-113).

Tank	Pathogen	Tissue Temp Fed	Number Stocked	Survivors Collected	% Survival	Survival by Group
1	Neg Control	None	15	0	0.0%	33.3%
2	Neg Control	None	15	10	66.7%	
3	WSSV	95 C	20	19	95.0%	90.0%
4	WSSV	95 C	20	17	85.0%	
5	WSSV	85 C	20	16	80.0%	90.0%
6	WSSV	85 C	20	20	100.0%	
7	WSSV	75 C	20	19	95.0%	97.5%
8	WSSV	75 C	20	20	100.0%	
9	WSSV	70 C	20	18	90.0%	75.0%
10	WSSV	70 C	20	12	60.0%	
11	WSSV	60 C	20	16	80.0%	82.5%
12	WSSV	60 C	20	17	85.0%	
13	WSSV Pos Control	Uncooked	20	0	0.0%	0.0%
14	WSSV Pos Control	Uncooked	20	0	0.0%	
15	Neg Control	Neg Control	15	13	86.7%	86.7%
16	Neg Control	Neg Control	15	13	86.7%	
17	YHV1	95 C	20	15	75.0%	80.0%
18	YHV1	95 C	20	17	85.0%	
19	YHV1	85 C	20	17	85.0%	90.0%
20	YHV1	85 C	20	19	95.0%	
21	YHV1	75 C	20	20	100.0%	97.5%
22	YHV1	75 C	20	19	95.0%	
23	YHV1	70 C	20	16	80.0%	85.0%
24	YHV1	70 C	20	18	90.0%	
25	YHV1	60 C	20	14	70.0%	80.0%
26	YHV1	60 C	20	18	90.0%	
27	YHV1 Pos Control	Uncooked	20	0	0.0%	0.0%
28	YHV1 Pos Control	Uncooked	20	0	0.0%	

Table 8. Summary of samples from the Case 22-113. Samples of juvenile shrimp *Penaeus vannamei* originated from the WSSV & YHV cooking study 04/21/2022 (Aranguren).

Tank# / treatment	Pathogen challenged	Sampling date mm/dd/yy	UAZ ID#	WSSV [1]	YHV[2]
Tank #B1 Case A	WSSV	**03/21/22 Day 3**	**22-113A/3**	**G4**[3]	
Tank #B1 Case A	WSSV	**03/21/22 Day 3**	**22-113A/4**	**G4**	
Tank #1 Case C	WSSV Negative control	**04/02'/22 Day 9**	**22-113C/1**	**ND**[4]	
Tank #2 Case D	WSSV Negative control	**04/07/22 Day 14**	**22-113D/1**	**ND**	
Tank #2 Case D	WSSV Negative control	**04/07/22 Day 14**	**22-113D/2**	**ND**	
Tank #3 Case E	95°C WSSV	**04/07/22 Day 14**	**22-113E/1**	**ND**	
Tank #3 Case E	95°C WSSV	**04/07/22 Day 14**	**22-113E/2**	**ND**	
Tank #4 Case F	95°C WSSV	**04/07/22 Day 14**	**22-113F/1**	**ND**	
Tank #4 Case F	95°C WSSV	**04/07/22 Day 14**	**22-113F/2**	**ND**	
Tank #5 Case G	85°C WSSV	**04/07/22 Day 14**	**22-113G/1**	**ND**	
Tank #5 Case G	85°C WSSV	**04/07/22 Day 14**	**22-113G/2**	**ND**	
Tank #6 Case H	85°C WSSV	**04/07/22 Day 14**	**22-113H/1**	**ND**	
Tank #6 Case H	85°C WSSV	**04/07/22 Day 14**	**22-113H/2**	**ND**	
Tank #7 Case I	75°C WSSV	**04/07/22 Day 14**	**22-113I/1**	**ND**	
Tank #7 Case I	75°C WSSV	**04/07/22 Day 14**	**22-113I/2**	**ND**	
Tank #8 Case J	75°C WSSV	**04/07/22 Day 14**	**22-113J/1**	**ND**	
Tank #8 Case J	75°C WSSV	**04/07/22 Day 14**	**22-113J/2**	**ND**	
Tank #9 Case K	70°C WSSV	**04/07/22 Day 14**	**22-113K/1**	**ND**	
Tank #9 Case K	70°C WSSV	**04/07/22 Day 14**	**22-113K/2**	**ND**	
Tank #10 Case K	70°C WSSV	**04/07/22 Day 14**	**22-113L/1**	**ND**	
Tank #10 Case K	70°C WSSV	**04/07/22 Day 14**	**22-113L/2**	**ND**	
Tank #11 Case M	60°C WSSV	**04/07/22 Day 14**	**22-113M/1**	**ND**	

Tank# / treatment	Pathogen challenged	Sampling date mm/dd/yy	UAZ ID#	WSSV [1]	YHV[2]
Tank #11 Case M	60°C WSSV	**04/07/22 Day 14**	**22-113M/2**	**ND**	
Tank #12 Case N	60°C WSSV	**04/07/22 Day 14**	**22-113N/1**	**ND**	
Tank #12 Case N	60°C WSSV	**04/07/22 Day 14**	**22-113N/2**	**ND**	
Tank #13 Case P	WSSV Positive control	**03/27/22 Day 3**	**22-113P/1**	**G4**	
Tank #13 Case P	WSSV Positive control	**03/27/22 Day 3**	**22-113P/2**	**G4**	
Tank #14 Case Q	WSSV Positive control	**03/26/22 Day 2**	**22-113Q/1**	**G4**	
Tank #14 Case Q	WSSV Positive control	**03/26/22 Day 2**	**22-113Q/2**	**G4**	
Tank #15 Case R	YHV Negative control	**04/14/22 Day 16**	**22-113R/1**		**ND**
Tank #15 Case R	YHV Negative control	**04/14/22 Day 16**	**22-113R/2**		**ND**
Tank #16 Case S	YHV Negative control	**04/14/22 Day 16**	**22-113S/1**		**ND**
Tank #16 Case S	YHV Negative control	**04/14/22 Day 16**	**22-113S/2**		**ND**
Tank #17 Case T	95°C YHV	**04/14/22 Day 16**	**22-113T/1**		**ND**
Tank #17 Case T	95°C YHV	**04/14/22 Day 16**	**22-113T/2**		**ND**
Tank #18 Case U	95°C YHV	**04/14/22 Day 16**	**22-113U/1**		**ND**
Tank #18 Case U	95°C YHV	**04/14/22 Day 16**	**22-113U/2**		**ND**
Tank #19 Case V	85°C YHV	**04/14/22 Day 16**	**22-113V/1**		**ND**
Tank #19 Case V	85°C YHV	**04/14/22 Day 16**	**22-113V/2**		**ND**
Tank #20 Case W	85°C YHV	**04/14/22 Day 16**	**22-113W/1**		**ND**
Tank #20 Case W	85°C YHV	**04/14/22 Day 16**	**22-113W/2**		**ND**
Tank #21 Case X	75°C YHV	**04/14/22 Day 16**	**22-113X/1**		**ND**
Tank #21 Case X	75°C YHV	**04/14/22 Day 16**	**22-113X/2**		**ND**
Tank #22 Case Y	75°C YHV	**04/14/22 Day 16**	**22-113Y/1**		**ND**
Tank #22 Case Y	75°C YHV	**04/14/22 Day 16**	**22-113Y/2**		**ND**
Tank #23 Case Z	70°C YHV	**04/14/22 Day 16**	**22-113Z/1**		**ND**
Tank #23 Case Z	70°C YHV	**04/14/22 Day 16**	**22-113Z/2**		**ND**

Tank# / treatment	Pathogen challenged	Sampling date mm/dd/yy	UAZ ID#	WSSV (1)	YHV(2)
Tank #24 Case AA	70°C YHV	**04/14/22 Day 16**	**22-113AA/1**		**ND**
Tank #24 Case AA	70°C YHV	**04/14/22 Day 16**	**22-113AA/2**		**ND**
Tank #25 Case BB	60°C YHV	**04/14/22 Day 16**	**22-113BB/1**		**ND**
Tank #25 Case BB	60°C YHV	**04/14/22 Day 16**	**22-113BB/2**		**ND**
Tank #26 Case CC	60°C YHV	**04/14/22 Day 16**	**22-113CC/1**		**ND**
Tank #26 Case CC	60°C YHV	**04/14/22 Day 16**	**22-113CC/2**		**ND**
Tank #27 Case DD	YHV positive control	**04/06/22 Day 8**	**22-113DD/1**		**G3**

Note:

1) WSSV =White spot syndrome virus
2) YHV= Yellow head virus
3) G-trace to G4=Severity grade of infection/lesion, according to enclosed severity grade table. Numbers on the left side of the parentheses indicate number of affected shrimp.
4) ND= Not detected

APPENDIX 1

Severity Grade	Clinical Findings
0	> No signs of infection/infestation by pathogen, parasite, or epicommensal present.
trace	> Signs of infection/infestation by pathogen, parasite or epicommensal are present at just above diagnostic procedure minimum detection limits.
1	> Signs of infection/infestation by pathogen, parasite or epicommensal are present, but at levels that may be below those needed for clinical disease. > Agent detected may be in early stages of infection and represent preclinical disease.
2	> Moderate signs of infection/infestation as shown by low to moderate numbers of parasite or epicommensal, or by number and severity of pathogen caused lesions. > Prognosis is for possible production losses and/or slight increases in mortality if no treatment (if treatable) or management change is applied.
3	> Moderate to high signs of disease apparent as shown by relatively higher numbers of parasite or epicommensal, or by number and severity of pathogen caused lesions. > Potentially lethal prognosis if no treatment (if treatable) or management change is applied.
4	> High numbers of parasite or epicommensal present, or for pathogen caused infections the presence of severe lesions and advanced tissue destruction. > Lethal prognosis, especially under conditions conducive to disease development (i.e. with low oxygen, ecdysis, changes in salinity or temperature, etc.).

Table 9. Time for frozen tissue to reach 60ºC, 70ºC, 75ºC, 85ºC, and 95ºC during WSSV and YHV1 cooking. (UAZ Case# 22-113)

WSSV Tissue		
Group	Internal Temp (C)	Time to Temp
WSSV 60 C	60	1:15
	59	1:30
	61	1:50
WSSV 70 C	70	1:34
	70	1:34
	70	2:05
WSSV 75 C	75	1:14
	74	1:22
	75	1:54
WSSV 85 C	84	1:25
	85	2:16
	85	2:40
WSSV 95 C	94	1:36
	95	2:15
	94	2:37
YHV1 Tissue		
Group	Internal Temp (C)	Time to Temp
YHV1 60 C	60	0:56
	60	1:08
	60	1:28
YHV1 70 C	70	0:51
	70	1:27
	70	1:27
YHV1 75 C	75	0:49
	75	1:16
	76	1:18
YHV1 85 C	85	0:57
	85	1:34
	85	2:12
YHV1 95 C	95	1:40
	95	2:22
	95	3:24