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# Thermal treatment increases survival of WSSV-infected shrimp

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## Maintaining constant water temperature crucial to reduce mortalities



Gas-powered heaters (left) provide warm water to raceways in a Peruvian shrimp farm. Electric heaters maintain water at  $32 \pm 1$  degrees C to control WSSV at a Thai farm (right).

In a study by the authors, Pacific white shrimp (*Litopenaeus vannamei*) collected from farms located in the Chantanburi Province of Thailand were infected with white spot syndrome virus (WSSV) by immersion and oral means to evaluate their related responses to water temperature.

The virus was kept at minus-80 degrees-C for reinfection of specific pathogen-free (SPF) *L. vannamei*. Quantified as pathogenic by real-time polymerase chain reaction (PCR), the inoculum was prepared from WSSV-infected tissues from the cephalothorax including gills and muscle. Prior to the experiments, SPF shrimp were randomly sampled and verified free of WSSV.

## WSSV challenges



Shrimp experimentally infected with WSSV showed the familiar white spots in various parts of their bodies.

In the immersion challenge, 180, 5-g shrimp were transferred into 21, 90-L aquariums equipped with aeration and heaters. Then 90 mL of the WSSV inocula were added to the aquarium water.

One group of shrimp was constantly maintained at  $32 \pm 1$  degrees-C, and a control group was held at  $28 \pm 1$  degrees-C after challenge. Other groups were kept at  $32 \pm 1$  degrees-C, with the water temperature reduced to  $28 \pm 1$  degrees-C at one, three, five and seven days post-challenge. Each group had three replicates.

For the oral route for challenge, another 180 shrimp were transferred into 21, 90-L aquariums with aeration and heating. Shrimp were initially fed once at 10 percent body weight with WSSV-infected shrimp and later fed twice daily with commercial pelleted feed at a rate of 5 percent body weight/day.

One group of shrimp was maintained at  $32 \pm 1$  degrees-C, and a control group was held at  $28 \pm 1$  degrees-C after challenge. Other groups were kept at  $32 \pm 1$  degrees-C, after which temperature was reduced to  $28 \pm 1$  degrees-C at one, three, five and seven days post-challenge. Each group had three replicates. Gross signs of disease and mortality were recorded every 12 hours until the end of the experiment. The status of moribund and surviving shrimp were confirmed by nested polymerase chain reaction testing and histopathology.

## Results of experiment

Shrimp constantly maintained at  $32 \pm 1$  degrees-C and those held at that temperature for seven days after challenge before switching to  $28 \pm 1$  degrees-C did not show clinical signs or mortality when challenged by either immersion or the oral route. Surviving shrimp were WSSV-negative by nested PCR as well as histopathology.

Initial mortalities were observed in the other groups of shrimp treated with immersion or oral challenge between 42 and 66 hours after challenge (Tables 1 and 2). Total mortality was observed between 114 and 168 hours.

### Limsuwan, Responses of healthy juvenile shrimp, Table 1

Temperature (°C)	Disease Signs Appear (hours)	Initial Mortality (hours)	Total Mortality (hours)	PCR Result
$24 \pm 1$	24	42	114	+
$28 \pm 1$	24	42	114	+
$32 \pm 1$	No sign	No mortality	No mortality	-

Table 1. Responses of healthy juvenile shrimp fed WSSV-infected shrimp.

### Limsuwan, Responses of healthy juvenile shrimp, Table 2

Temperature (°C)	Disease Signs Appear (hours)	Initial Mortality (hours)	Total Mortality (hours)	PCR Result
$24 \pm 1$	36	54	144	+
$28 \pm 1$	36	66	168	+
$32 \pm 1$	No sign	No mortality	No mortality	-

Table 2. Responses of healthy juvenile shrimp immersed in WSSV-infected water.

## Thermal treatment increased survival

Solving problems related to water temperature in grow-out ponds to control WSSV is almost impossible, even with the use of greenhouses. On the other hand, during larval rearing in hatcheries, temperatures of  $32 \pm 1$  degrees-C can easily be maintained using heaters. So far, not many hatcheries elevate water temperatures to these levels. Instead, they normally maintain 28 to 30 degrees-C or even lower in those hatcheries that do not have heaters during winter periods.

In spite of having PCR detection of WSSV for postlarvae before stocking, many farms in Latin American and Thailand found the larvae samples were not always fully representative of the stocked population, and WSSV outbreaks would occur when temperatures dropped below 30 degrees-C. Even when biosecurity measures were employed in conjunction with zero water exchange during the first 60 days after stocking, WSSV mortalities always occurred. Some of the farms surveyed decided to apply the thermal treatment of  $32 \pm 1$  degrees-C for seven days, which resulted in significantly increased survival rates (Table 3).

## Limsuwan, Final survival rates for farm ponds, Table 3

Farm Location	Survival in Ponds Without Thermal Treatment (%)	Survival in Ponds With Thermal Treatment (%)
Ecuador	40-50	50-75
Peru	35-45	60-80
Thailand	30-50	50-70

Table 3. Final survival rates for farm ponds located in WSSV-infected areas.

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