Effect of high water temperature (33 °C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei* 

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## Abstract

White spot syndrome virus (WSSV) is the most lethal pathogen of cultured shrimp. Previous studies done with undefined WSSV titers showed that high water temperature (32–33 °C) reduced/delayed mortality of WSSV-infected shrimp. This study evaluated the effect of high water temperature on the clinical and virological outcome of a WSSV infection under standardized conditions. Groups of specific pathogen-free Litopenaeus vannamei were challenged either by intramuscular or oral routes with a low (30 SID50) or a high (10,000 SID50) virus titer. Shrimp were kept (i) continuously at 27 °C, (ii) 30 °C or (iii) 33 °C; (iv) maintained at 33 °C before challenge and 27 °C afterwards, or (v) kept at 27 °C before challenge and 33 °C afterwards. Shrimp were maintained at the respective temperatures for 120 h before challenge and 120–144 h post challenge (hpc). Gross signs and mortality were monitored every 12 h until the end of the experiment. Dead and surviving shrimp were screened for WSSV infection (VP28-positive cells) by indirect

immunofluorescence (IIF). Shrimp kept continuously at 27 °C or 30 °C, or switched to 27 °C post challenge developed gross signs within 24 hpc, first mortalities at 36-60 hpc and 100% cumulative mortality between 60 and 144 hpc depending on the virus titer. All dead shrimp were WSSV-positive. In contrast, shrimp kept at 33 °C continuously or after WSSV challenge showed no signs of disease and low mortalities (0-30%) regardless of the virus titer. Dead and surviving shrimp were WSSV-negative. Further, early virus replication was studied in two groups of shrimp: one maintained at 27 °C before and after challenge and one switched from 27 °C to 33 °C after challenge with 10,000 SID50. Immunohistochemistry (IHC) analysis showed that WSSV-positive cells were first displayed at 12 hpc in shrimp kept at 27 °C and by 24 hpc the infection became systemic. In contrast, shrimp kept at 33 °C did not display WSSV-positive cells at 12 or 24 hpc. This work confirms previous reports that high water temperature prevents the onset of disease and significantly reduces mortality of WSSV-inoculated shrimp regardless of the route of inoculation or virus titer used. This strategy may have practical applications to control WSSV in tropical shrimp farming countries.

## Keywords

WSSV replication; VP28; Water temperature; Litopenaeus vannamei; SPF