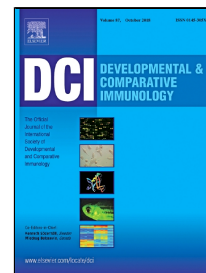


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**The effect of temperature on White Spot Disease progression in a crustacean,
*Pacifastacus leniusculus****

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Abstract

The effects of temperature on the progression of White Spot Disease (WSD) have been studied in the freshwater crayfish *Pacifastacus leniusculus*. In this study, we aimed to understand the reason for previously observed low mortalities with white spot syndrome virus (WSSV) infected crayfish at low temperatures. The susceptibility of freshwater crayfish to WSSV was studied at different temperatures. The mortality rate at 6°C was zero, meanwhile the animals kept at 22°C developed WSD symptoms and died in a few days after WSSV injections, however upon transfer of animals from 6°C to 22°C the mortality reached 100% indicating that the virus is not cleared. Moreover, the VP28 expression at 6°C was significantly lower compared to animals kept at 22°C. We injected animals with demecolcine, an inhibitor that arrests the cell cycle in metaphase, and observed a delayed mortality. Furthermore, the VP28 expression was found to be lower in these animals receiving both injections with WSSV and demecolcine since cell proliferation was inhibited by demecolcine. We quantified WSSV copy numbers and found that virus entry was blocked at 6°C, but not in demecolcine treatments. We supported this result by quantifying the expression of a clip domain serine protease (*PlcSP*) which plays an important role for WSSV binding, and we found that the *PlcSP* expression was inhibited at 6°C. Therefore, our hypothesis is that the

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