

**TaqMan real-time PCR assay for relative quantitation of white spot syndrome virus infection in *Penaeus monodon* Fabricius exposed to ammonia.**

ABSTRACT

White spot disease is caused by a highly virulent pathogen, the white spot syndrome virus (WSSV). The disease is usually triggered by changes in environmental parameters causing severe losses to the shrimp industry. This study was undertaken to quantify the relative WSSV load in shrimp exposed to ammonia, using a TaqMan-based real-time PCR, and their subsequent susceptibility to WSSV. Shrimp were exposed to different levels of total ammonia nitrogen (TAN) (8.1, 3.8 and 1.1 mg L<sup>-1</sup>) for 10 days and challenged with WSSV by feeding WSSV-positive shrimp. WSSV was detected simultaneously in haemolymph, gills and pereopods at four hours post-infection. The TaqMan real-time PCR assay showed a highly dynamic detection limit that spanned over 6 log<sub>10</sub> concentrations of DNA and high reproducibility (standard deviation 0.33–1.42) and small correlation of variability (CV) (1.89–3.85%). Shrimp exposed to ammonia had significantly higher ( $P < 0.01$ ) WSSV load compared to the positive control, which was not exposed to ammonia. Shrimp exposed to 8.1 mg L<sup>-1</sup> of TAN had the highest ( $P < 0.01$ ) WSSV load in all three organs in comparison with those exposed to 3.8 and 1.1 mg L<sup>-1</sup> of TAN. However, haemolymph had significantly higher ( $P < 0.01$ ) viral load compared to the gills and pereopods. Results showed that shrimp exposed to ammonia levels as low as 1.1 mg L<sup>-1</sup> (TAN) had increased susceptibility to WSSV.

**Keyword:** White spot syndrome virus; Ammonia; Relative quantification; TagMan real time PCR.