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Short-term hyperthermic treatment of *Penaeus monodon* increases expression of heat shock protein 70 (HSP70) and reduces replication of gill associated virus (GAV)

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Abstract

Disease is the result of interactions amongst pathogens, the environment and host organisms. To investigate the effect of stress on *Penaeus monodon*, juvenile shrimp were given short term exposure to hypoxic, hyperthermic and osmotic stress twice over a 1-week period and estimates of total haemocyte count (THC), heat shock protein (HSP) 70 expression and load of gill associated virus (GAV) were determined at different time points. While no significant differences were observed in survival and THC between stressed and control shrimp ($P > 0.05$), HSP 70 expression and GAV load changed significantly ($P < 0.05$). HSP 70 expression was higher in the hyperthermic treatment than in other treatments ($P < 0.05$). GAV load increased throughout the 21-day experiment for all groups and treatments except for hyperthermic stress. These findings suggest that a beneficial reduction in the rate of GAV replication, which cannot simply be attributed to holding the animals at a non-permissive temperature for GAV, results from short-term hyperthermic treatment.

Keywords: *Penaeus monodon*; Gill-associated virus; Environmental stress; Heat shock protein; Immune parameters

1. Introduction

The health of aquatic species is influenced by interactions between the environment, pathogens and the host. Under intensive shrimp aquaculture, environmental conditions can degrade rapidly causing significant stress to the shrimp and reducing their fitness (Capy et al., 2000). This may make them more susceptible to diseases (Lee and Wickins, 1992). Alternatively, environmental changes may result in activation of pathogenic gene pathways such that an otherwise benign pathogen causes disease (Miller et al., 2005). In either case, environmental conditions can be decisive in the progression from infection to disease (Hall and van Ham, 1998; Le Moullac et al., 1998; Lightner and Redman, 1998; Sanchez et al.,

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2001; Hall and de la Vega, 2004). In shrimp production systems many potential pathogens, such as bacteria, fungi and viruses, co-exist with shrimp without causing a negative impact on production (Flegel and Pasharawipas, 1998; Spann et al., 2000; Vidal et al., 2001). However, some quiescent viral infections may develop into acute diseases if shrimp become stressed and this has repeatedly lead to significant industry losses (Cowley et al., 2000; Vidal et al., 2001; Hall and de la Vega, 2004). For industry to develop strategies to these risks a better understanding of the relationship between environmental stress, immunological vigour and epizootics is required.

In marine crustaceans, knowledge of this relationship is limited, but increasing (Direkbusarakom and Danayadol, 1998; Hall and van Ham, 1998; Le Moullac et al., 1998, Le Moullac and Haffner, 2000; Perazzolo et al., 2002). Multiple studies have described the effects of environmental factors such as water temperature, salinity, oxygen, ammonia and nitrite levels on immuno-parameters such as haemocyte counts, antibacterial and phagocytic activity, release of reactive oxygen intermediates and modulation of the prophenoloxidase activity (Le Moullac et al., 1997, 1998; Le Moullac and Haffner, 2000; Sanchez et al., 2001; Pascual et al., 2002; Perazzolo et al., 2002; Liu et al., 2003; Jiang et al., 2005). Most studies have focused on bacterial diseases and anti-bacterial responses. Work on environmentally induced viral diseases is very limited. Bray et al. (1994) described a relationship between salinity and infectious hypodermal and haematopoietic necrosis virus (IHHNV) in the Pacific White shrimp, *Litopenaeus vannamei*. More recently, there have been reports on the effect of water temperature on the pathogenicity of white spot syndrome virus (Vidal et al., 2001; Granja et al., 2003; Guan et al., 2003).

Gill associated virus (GAV) is a virulent pathogen of *Penaeus monodon* that is thought to be responsible for production losses in Australia since 1996 (Spann et al., 1997, 2000). Subclinical or chronic GAV infections are highly prevalent in wild and farmed *P. monodon* along the East Coast of Australia (Spann et al., 1995; Walker et al., 2001; Cowley et al., 2002) and are characterized by restriction of GAV primarily to spheroid bodies within the lymphoid organ. By contrast other tissue types are involved in acute infections associated with morbidity and mortality (Smith, 2000; Spann et al., 2000, 2003; Tang et al., 2002). The environmental stresses and physiological mechanisms that trigger transition from the chronic to acute state remain poorly understood. A recently developed quantitative real time RT-PCR assay for GAV has enabled us to observe high levels of viral replication in shrimp subject to handling stress (de la Vega et al., 2004). In this report this technique has been used to analyze the effect of other types of stress on GAV replication and disease.

Stress classically leads to a rapid cascade of molecular and physiological responses that can be evaluated by molecular and biochemical indicators (Sanchez et al., 2001). Heat-shock proteins (HSPs) are key indicators and comprise a group of highly conserved proteins that have general protective functions in all living organisms. They act as molecular chaperones that regulate protein homeostasis and refolding of unfolded proteins after partial denaturation (Lindquist, 1986; McLennan and Miller, 1990). In addition, they have been linked to inhibition of viral replication (Demarco and Santoro, 1993; Santoro, 1994, 1997; Virgilio et al., 1997, Conti et al., 1999). Since environmental stresses induce their expression, they are biomarkers for stress events (Tirard et al., 1995; Lewis et al., 1999). HSP induction by stress has been documented in shrimp (Downs et al., 2001; Cimino et al., 2003) but the relationship between HSP induction and shrimp health has not been studied.

To further understand the effect of environmental stress on the shrimp immune system and GAV replication and disease, we exposed juvenile *P. monodon* to three types of environmental stress, and measured changes in GAV load, total haemocyte counts and expression of heat shock proteins.

2. Materials and methods

2.1. Animals and experimental design

Juvenile tiger shrimp (average weight 15.9 g) were collected from a local shrimp farm and transferred to the Australian Institute of Marine Science (AIMS) hatchery in Townsville, Australia. Healthy-looking animals (180) at the intermolt stage were transferred and acclimated for 5 days in twelve 100 L tanks (15