EFFECT OF LOW WATER TEMPERATURE ON THE PATHOGENICITY OF WHITE SPOT SYNDROME VIRUS (WSSV) IN KURUMA SHRIMP (*Marsupenaeus japonicus)*

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ABSTRACT

White spot syndrome virus (WSSV) is a highly lethal, stress-dependent virus which causes serious economic losses for shrimp farming worldwide. Measures that boost/stimulate the shrimp immune system to control WSSV are not yet available and, therefore, environmental management to minimize stress plays a major role in disease prevention. This study was performed to investigate the effect of water temperature on WSSV infectivity, and to evaluate the effect of low temperature on pathogenicity of WSSV in kuruma shrimp, *Marsupenaeus japonicus*. The results showed that the earliest and highest mortality patterns, culminating with 100% mortalities at 7 d.p.c., were observed when shrimp was continuously kept at 25^{o}C, followed by those of shrimp was continuously kept at temperature of 20^{o}C. The best survival (80%) was observed when shrimp continuously kept at 15^{o}C. The delayed and reduced mortalities were observed when shrimp were transferred from 25^{o}C to 15^{o}C compared to shrimp held at 25^{o}C before and after WSSV challenge. In contrast, the increased mortalities were observed in shrimp shifted from 15^{o}C to 25^{o}C when compared to mortalities of shrimp continuously held at 15^{o}C. PCR and RT-PCR provided evidences confirming and supporting the mortality assay. This study shows that WSSV infection in kuruma shrimp is temperature dependent and shrimp was highly susceptible to WSSV infection at around 25^{o}C. Low temperature (15^{o}C) reduces rather than stop WSSV replication in infected shrimp. Shrimp at 15^{o}C may act a carrier of WSSV and could spread the disease if water temperature is increased.

Keywords: Challenge, WSSV, mortality, shrimp, temperature.

Nghiên cứu ảnh hưởng của nhiệt độ nước thấp đến sự gây bệnh của vi rút đốm trắng (WSSV) trên tôm he Nhật Bản (*Marsupenaeus japonicus)*

Vi rút đốm trắng (WSSV) là vi rút gây bệnh có độc lực mạnh trên tôm nhưng dễ bị ảnh hưởng bởi các yếu tố gây sốc. Các biện pháp khống chế WSSV đã và đang được nghiên cứu nhưng vẫn chưa được áp dụng vào thực tiễn, do vậy việc quản lý môi trường nhằm hạn chế các yếu tố gây sốc đóng vai trò quan trọng trong việc ngăn ngừa bệnh WSSV. Nghiên cứu này đánh giá ảnh hưởng của nhiệt độ nước, đặc biệt là nhiệt độ nước thấp, đến khả năng gây bệnh của WSSV trên tôm he Nhật Bản. Kết quả nghiên cứu cho thấy tỷ lệ tôm chết sớm và cao nhất, lên tới 100% sau 7 ngày gây nhiễm WSSV, đã quan sát thấy ở nghiệm thức tôm nuôi ở 25^{o}C, tiếp đến là ở nghiệm thức tôm nuôi ở 20^{o}C và tỷ lệ chết thấp nhất ở nghiệm thức tôm được giữ ở 15^{o}C. Nghiên cứu cũng cho thấy, tỷ lệ tôm chết xuất hiện muộn và thấp hơn ở tôm thí nghiệm nuôi ở 25^{o}C nhưng được hạ nhiệt độ xuống 15^{o}C sau khi gây nhiễm khi so với tôm trước và sau khi gây nhiễm đều giữ ở 25^{o}C. Ngược lại, tỷ lệ tôm chết tăng lên ở nghiệm thức tôm giữ ở 15^{o}C sau đó được nâng lên 25^{o}C sau khi gây nhiễm khi so với nghiệm thức tôm giữ ở 15^{o}C trước và sau khi gây nhiễm. Kết quả này được kiểm chứng bằng kỹ thuật PCR và RT-PCR. Kết quả nghiên cứu cho thấy sự gây bệnh của WSSV trên tôm he Nhật Bản phụ thuộc vào nhiệt độ và tôm mẫn cảm nhất với WSSV ở nhiệt độ khoảng 25^{o}C. Nhiệt độ thấp (khoảng 15^{o}C) đã làm chậm sự nhân lên của WSSV trên tôm nhiễm bệnh và ở nhiệt độ này tôm được xem như là nguồn mang mầm bệnh WSSV tiềm ẩn, khi nhiệt độ nước tăng lên bệnh sẽ bùng phát.

Từ khóa: Cảm nhiễm, nhiệt độ, tôm, tỷ lệ chết, vi rút đốm trắng, WSSV.

1. INTRODUCTION

White spot syndrome virus (WSSV) is an enveloped, non-occluded and rod shaped baculovirus which is recently classified as the sole member of the genus *Whispovirus* and family *Nimaviridae* (Mayo, 2002a; Mayo, 2002b). Since first appearing in the 1990s in Asia, WSSV has quickly spread to the whole world and become one of the most devastating pathogens of the shrimp industry. The virus can infect shrimp at any stage in its life cycle and target almost all organs/tissues of the host. An acute outbreak of the disease can cause a cumulative mortality of up to 100% within 3-10 days (Escobedo-Bonilla et al., 2008; Leu et al., 2009; Sanchez-Martinez, 2007; Xu et al., 2009). In addition, WSSV is capable of producing a persistent infection in the host and at least 78 species of crustaceans have been reported as hosts or carriers of the WSSV either from culture facilities, wild, or experimentally infected animals (Escobedo-Bonilla et al., 2008). The virus is never completely eliminated and it resurfaces, particularly at times of stress, to cause mortalities. Thus, frequent WSSV disease outbreaks still occur worldwide and strategies for prevention or control of the disease are major challenges for shrimp industries.

Disease is the end result of complex interactions between host, pathogen and environment (Lightner and Redman, 1998). Unlike vertebrate immunity which is composed of both innate and adaptive responses, shrimp lacks adaptive immunity, and it relies on innate defense system to combat infections. In addition, the virus lacks many of pattern molecules found in bacteria and fungi that stimulate immune responses (Johnson et al., 2008). In this context, the most successful strategies for prevention or control of viral diseases in shrimp are based on proper environmental management to minimize stress.

Sudden changes in environmental conditions trigger viral diseases, and several reports about temperature dependence of viral diseases in aquatic animals are available (Jiravanichpaisal et al., 2004). Some studies on the effect of temperature on the outcome of WSSV infections in crustaceans have been already documented, and it is widely assumed that temperature plays an important role in inducing outbreaks of WSSV disease (Du et al., 2008). Although, both hyperthermia and hypothermia have been reported to effect on WSSV infections in shrimp and crayfish, the effects greatly vary between the life stages of the host as well as the species (Du et al., 2008; Granja et al., 2006; Jiravanichpaisal et al., 2004; Rahman et al., 2006; Reyes et al., 2007). Also, little is known whether shrimp could act as a carrier of WSSV at low or high temperature. The present study was carried out to (1) investigate the effect of specific water temperatures on the susceptibility of kuruma shrimp (*M. japonicus*) to WSSV infection, and (2) evaluate the effect of low temperature on the pathogenicity of WSSV in *M. japonicus*.

2. MATERIALS AND METHODS

2.1. Virus stock and virus inoculum

WSSV stock is prepared as mentioned in our previous study (Dang et al., 2010). The experimental viral inoculum was also prepared following Dang et al. (2010) in order to obtain optimal responses for testing the effect of temperature on WSSV infection, and to collect samples of both dead and surviving challenged-shrimp after 10 days of infection for PCR and RT-PCR assays.

Experimental kuruma shrimp

Kuruma shrimp, *M. japonicus*, of approximately 10 g body weight, were purchased from a commercial shrimp farm (Miyazaki, Japan). The shrimp were acclimated and reared in a re-circulating water tank system maintained at about 15°C - 20°C and 30 ppt salinity prior to the experiments. The WSSV-free status of randomly selected samples of the experimental shrimp was confirmed both by Shrimple WSSV kit (EnBioTec Laboratories Co. Ltd., Japan) and PCR assay using gene-specific primers amplifying the full-length of *Wssv*VP28 (Table 1) before experimental challenge.

Table 1. Primers used for PCR and RT-PCR analysis

Primer name	Oligonucleotide sequence (5'-3')	Sequence position	PCR (RT-PCR) product (bp)
*Wssv*VP28-F	ATGGATCTTTCTTTCACTCTTTC	Full-length	615
*Wssv*VP28-R	TTACTCGGTCTCAGTGCCAG		
*Wssv*ORF332-F	CCTGACCACATCAAGAGGGT	1173	539
*Wssv*ORF332-R	TCGTTGATGGGTGTTGAAGA	1711	
*Wssv*ORF172-F	TCGACTGTGAACTGGACAGC	176	453
*Wssv*ORF172-R	CATGCCTACTGCATCCACTG	628	
*Wssv*ORF188-F	AAGAGGATTGCCCAGGAAGT	100	463
*Wssv*ORF188-R	GGAAGTGTTGTGCAGCGTTA	562	
*Wssv*ORF395-F	ATATGTGCTTTCCCGACAGG	704	454
*Wssv*ORF395-R	GTTTGCACCTCCTCAATGGT	1157	
*Wssv*ORF514-F	TCACGTGATTAGTGGGTGGA	1875	450
*Wssv*ORF514-R	TAGGCCTTTTCGCACACTTT	2324	
[a] EF1α-F	ATGGTTGTCAACTTTGCCCC	-	500
EF1α-R	TTGACCTCCTTGATCACACC		
[b] DecOIE-F	TGCCTTATCAGCTNTCGATTGTAG	-	848
DecOIE-R	TTCAGNTTTGCAACCATACTTCCC		

Note: [a] EF-1α specific primers were taken from a previous publication (Dang et al.*, 2010)*

[b] Forward and reverse decapod-specific primers corresponding to 143F and 145R, respectively, were taken from OIE website (http://www.oie.int/eng/normes/fmanual/A_00048.htm, manual of diagnostic tests for Aquatic Animals, 2003).

Effect of temperature on susceptibility of kuruma shrimp to WSSV

Shrimp were tested at 4 different specific temperatures, including 15°C, 20°C, 25°C, and 33°C. Prior to WSSV challenge, shrimp were exposed to 4 specific mentioned-above temperatures for at least 1 day. After that, each group of 10 shrimp was injected intramuscularly with 0.1 ml of 10^4x diluted WSSV stock. At each indicated temperature, the control group was injected with 0.1 ml of PBS (Phosphate Buffered Saline). Treated shrimp were continuously maintained at specific temperatures. The number of dead shrimp was recorded daily up to 10 d.p.c. (days post challenge) for the cumulative mortality assay. The experiment was conducted induplicate. The presence of WSSV in freshly dead shrimp was confirmed by PCR assay.

Effect of low temperature (15°C) on WSSV pathogenicity

The experiment was designed as shown in Fig. 1. Briefly, shrimp were exposed to 15°C and 25°C for 1 day prior to WSSV challenge, and were then divided into groups: (i) maintained at 15°C before challenge and 25°C afterwards, (ii) maintained at 25°C before challenge and 15°C afterwards, (iii) continuously maintained at 15°C, and (iv) continuously maintained at the 25°C. At each indicated temperature, PBS-injected shrimp were also included to serve as control groups. The number of dead shrimp was recorded daily for the cumulative mortality assay up to 10 days post challenge.

PCR and RT-PCR analysis for effect of water temperature on WSSV replication

Shrimp exposed to 4 specific temperatures (15°C, 20°C, 25°C, and 33°C) were injected with WSSV (10^4 x diluted stock) and then continuously kept at each specific temperature for the time-course DNA and RNA sampling. At various times post-challenge (3, 5, and 10 d.p.c.), total DNA and RNA were extracted from the gills of two surviving shrimp selected randomly from each experimental group and subjected to PCR and RT-PCR analysis using gene-specific primers shown in Table 1.

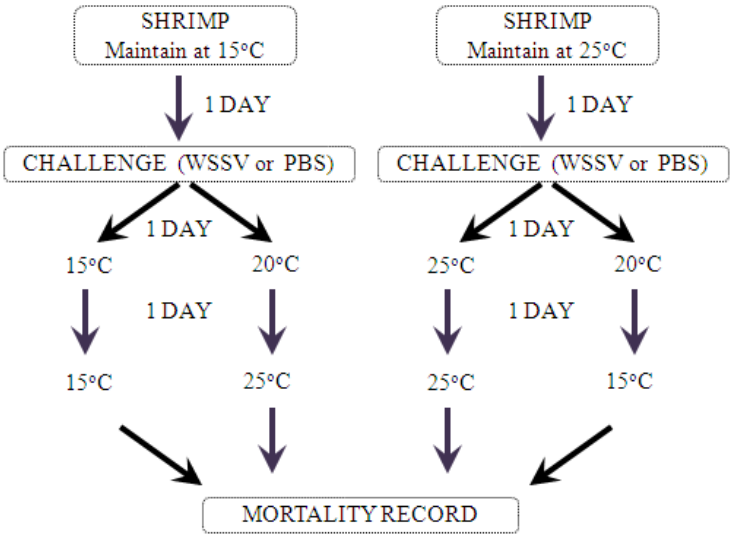


Fig. 1. Diagram of experimental design

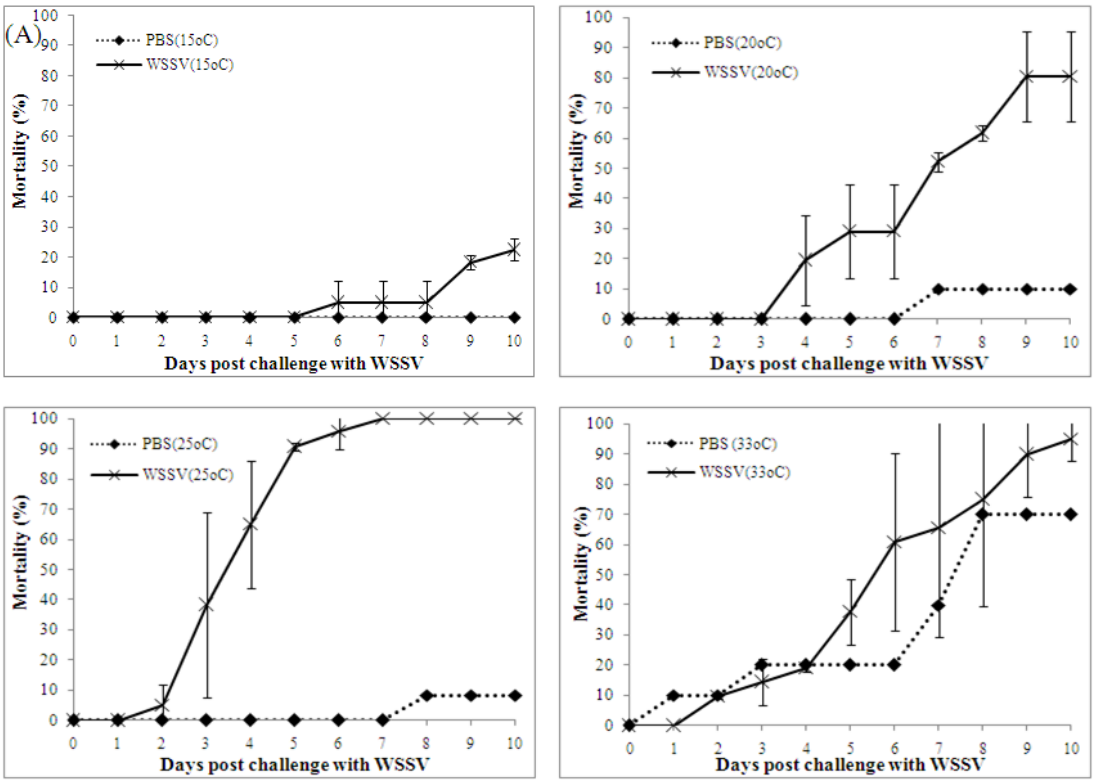
Total DNA was extracted with a ZR viral DNA kit (Zymo Research Corp., USA) following the manufacturer's protocol and equal amounts of DNA were used as templates for PCR analysis to detect WSSV loads in surviving challenged-shrimp. PCR was performed using the following protocol: 5 min at 95°C, followed by 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min.

Total RNA was extracted with RNAiso (Takara Bio Inc., Japan), treated with RNAse-free DNase I (Promega, USA), and then reverse transcribed to cDNAs. Equal amounts of cDNA were used as templates for RT-PCR analysis in order to determine the expression levels of some WSSV transcripts, known to play important roles during the virus propagation in infected host. These included *Wssv*VP28 protein that is especially involved in the attachment to, and penetration of, WSSV into host cells (Escobedo-Bonilla et al., 2008; van Hulten et al., 2001), *Wssv*ORF332 that is classified as a latency related-gene especially associated with persistent/latent WSSV infections (Dang et al., 2010), and 4 other WSSV ORFs that encode enzymes involved in viral DNA replication, namely (1) *Wssv*ORF172 (Ribonucleotide reductase large submit 1), (2) *Wssv*ORF188 (Ribonucleotide reductase large submit 2), (3) *Wssv*ORF395 (Thymidine and thymidylate kinase), and (4) *Wssv*ORF514 (DNA polymerase) (Leu et al., 2009). RT-PCR was performed using the following protocol: denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and extension at 72°C for 1 min.

3. RESULTS

3.1. Effect of temperature on susceptibility of kuruma shrimp to WSSV

Shrimp kept continuously at 25°C displayed the earliest and highest mortality pattern. The first mortality was observed at 2 d.p.c., high mortality occurred during 3-6 d.p.c., and reached cumulative mortality of 100% by 7 d.p.c. Lower temperature (20°C) delayed/reduced mortalities and the best survival rate (80%) was observed in shrimp maintained at 15°C. Although high mortalities were observed in WSSV-challenged shrimp maintained at 33°C, a similar observation was detected for the control group injected with PBS and maintained at 33°C (Fig. 2A). The results suggest that WSSV infection, in kuruma shrimp, is temperature dependent. Shrimp were highly susceptible to WSSV infection at around 25°C. Low temperature (15°C) reduced the spread of WSSV, suggesting a decrease in mortality during the virus challenge. PCR



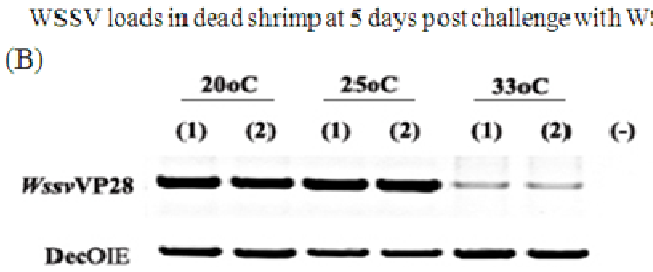


Fig. 2. Assay for effect of temperature on susceptibility of *M. japonicas* to WSSV. (A) Cumulative mortality of shrimp infected with WSSV at different specific temperatures; (B) PCR analysis of WSSV loads in WSSV challenged-shrimp

Note: (Line 1) The full-length of WssvVP28; (Line 2) DecOIE as a reference gene. Lanes (1) and (2) represent DNA from a single shrimp, and lane (-) indicates negative control sample (water template).

analysis revealed a distinct band of the full-length of *Wssv*VP28 from freshly dead challenged-shrimp, in which a slight band was observed in shrimp kept at 33°C (Fig. 2B).

3.2. Effect of low temperature on WSSV pathogenicity

Shrimp kept continuously at 15°C did not show mortality during the first 8 days post-challenge with WSSV and had cumulative mortality of about 20% at the end of the experiment. However, mortality recorded at day 3 in shrimp switched from 15°C to 25°C after challenge reached about 90% between 6-10 d.p.c. In contrast, shrimp kept continuously at 25°C first showed mortality at day 2 and displayed cumulative mortality of 100% at 7 d.p.c. Interestingly, the delayed and reduced

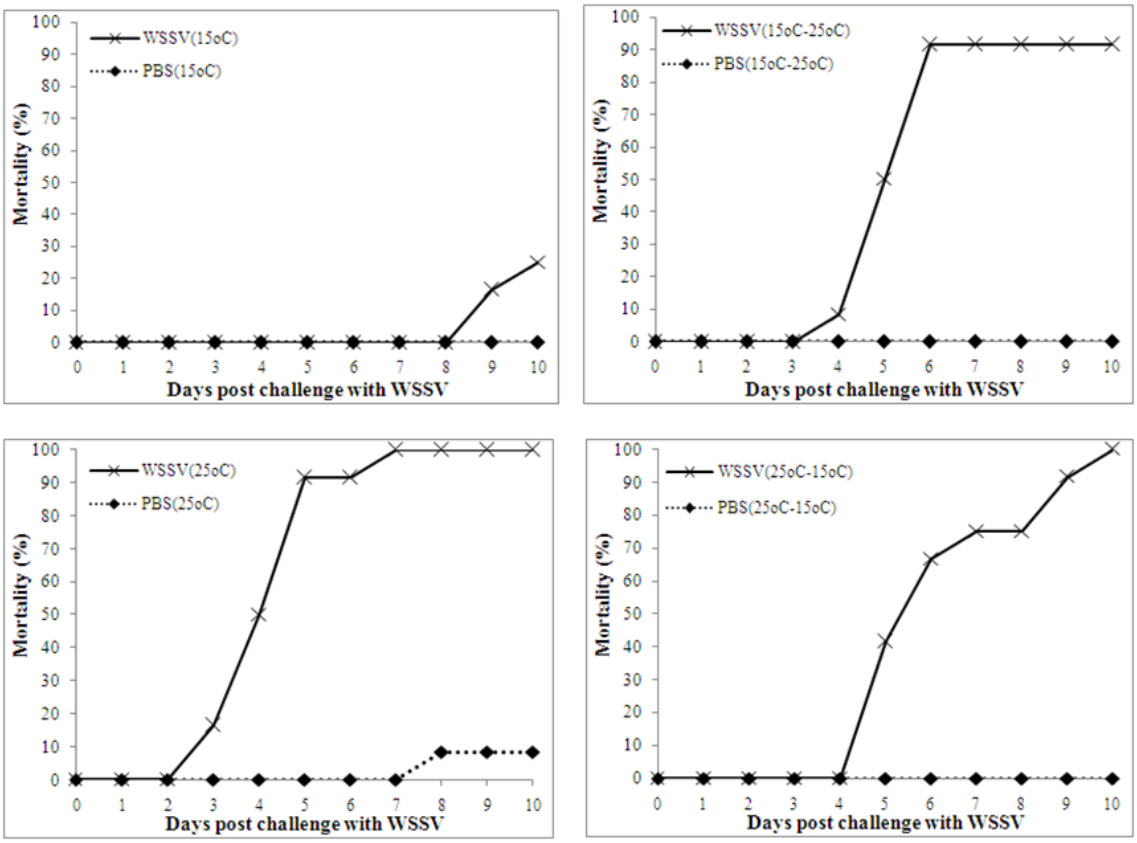


Fig. 3. Cumulative mortality analysis at assay for effect of low temperature (15°C) on WSSV pathogenicity in *M. japonicus*

mortality was observed in shrimp switched from 25°C to 15°C after challenge, with the first mortality at day 4 and the cumulative mortality of 100% at 10 d.p.c. (Fig. 3). These results indicated that low temperature (15°C) affects the pathogenicity of WSSV. It reduced rather than stopped WSSV replication in infected shrimp.

3.3. PCR and RT-PCR analysis

PCR analysis showed that high viral loads were found from samples taken from infected shrimp kept at 25°C throughout the challenge (Fig. 4A). While low viral loads were observed in shrimp kept at 15°C or 20°C at the early period of challenge (3 d.p.c), they seem to increase afterwards (5 and 10 d.p.c.). In contrast, a higher viral load was detected in shrimp kept at 33°C and at 3 d.p.c. compared to that of shrimp kept at 33°C and at 10 d.p.c.

RT-PCR analysis showed that expressions of viral ORFs that are involved in viral replication were under detectable levels in shrimp kept at 15°C (Fig. 4B).

4. DISCUSSION

Shrimp possess only an innate immune system, not an adaptive immune system, which responds to infections. Therefore, prevention of viral diseases is a major challenge to shrimp farming worldwide. Although several strategies that boost and stimulate shrimp immunity, such as vaccination, and use of immune stimulants and probiotics to control WSSV, to date, no adequate treatments are available to effectively control WSSV in the field (Bui, 2010; Sanchez-Martinez, 2007). In this context, the development of good management practices, the control of environmental variables to minimize stress, plays a major role in the control of WSSV disease.

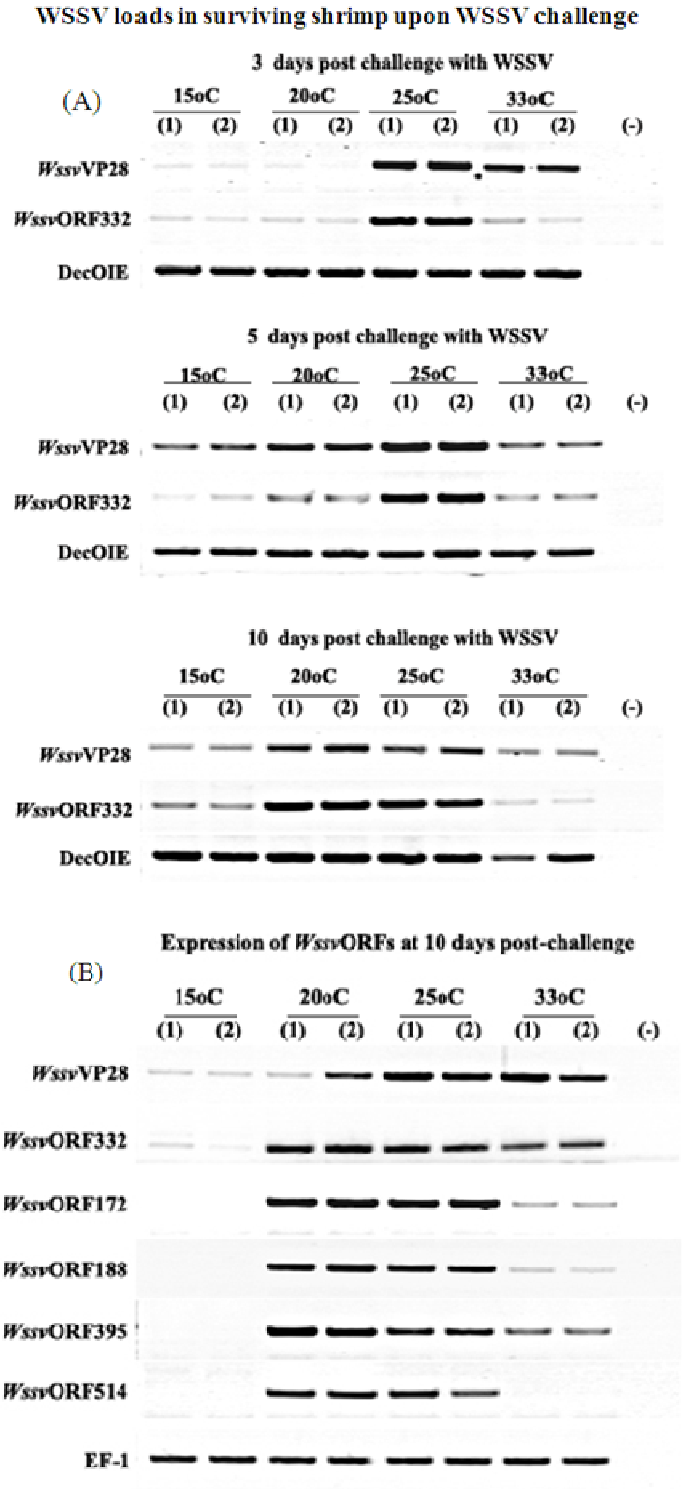


Fig. 4. Assay for effect of water temperature on WSSV replication in *M. japonicas*. (A) PCR analysis of WSSV loads in WSSV challenged-shrimp; (B) RT-PCR analysis of expression of WSSV transcripts in WSSV challenged-shrimp

Note: WssvVP28 (Line 1); WssvORF332 (Line 2); WssvORF172 (Line 3); WssvORF188 (Line 4); WssvORF395 (Line 5); WssvORF514 (Line 6); DecOIE and EF-1α as reference genes. Lanes (1) and (2) represent DNA or cDNA from a single shrimp, and lane (-) indicates negative control sample (water template).

The effects of different water temperatures on the progress of WSSV in aquatic invertebrates have been reported by some studies (Du et al., 2008; Granja et al., 2006; Rahman et al., 2006; and Reyes et al., 2007). Although both hypothermia and hyperthermia have been demonstrated to affect WSSV pathogenicity and mortality rate in crayfish and shrimp, results differ from species (Du et al., 2008; Granja et al., 2006; Jiravanichpaisal et al., 2004; Rahman et al., 2006). Because optimum temperatures for the growth of kuruma shimp range between 25°C and 30°C and the kuruma shrimp stop feeding at 15°C (https://www.business.qld.gov.au/industry/fisheries/aquaculture), four different specific temperature, including 15°C, 20°C, 25°C and 33°C were tested in this study to investigate the effect of water temperatures on the susceptibility of kuruma shrimp to WSSV infection. According to a previous study, WSSV infection in kuruma shrimp is temperature dependent, in which lower temperatures could reduce mortality rate (Guan et al., 2003). Our results are in agreement with findings found by Guan et al. (2003).

It has been also reported that temperatures between 16°C and 32°C allow WSSV replication in susceptible hosts such as shrimp, crabs and crayfish (Jiravanichpaisal et al., 2004; Jiravanichpaisal et al., 2006; Rahman et al., 2006). From our results, the favourable temperature range for WSSV replication is around 25°C, at which the earliest and highest mortality (Fig. 2A), the highest WSSV load (Fig. 2B, 4A) and the highest replication rate (Fig. 4B) were detected. By contrast, 15°C is outside the optimum range, the progress of WSSV disease spreads slower, showing the highest shrimp survival (Fig. 2A) and less replication of the virus (Fig. 4B).

Our results also show that shrimp were constantly dead throughout the experiment, and ended with high mortalities in both WSSV-challenged and PBS-infected shrimp group at 33°C (Fig. 2A), and that a low WSSV load was detected in dead shrimp kept at 33°C (Fig. 2B). A disadvantage of high temperature is the negative influence on other environmental variables such as levels of dissolved oxygen, evaporation rate, salinity and concentration of toxic metabolisms such as ammonia and nitrites, which are all critical for the normal shrimp metabolism (de la Vega et al., 2007a; de la Vega et al., 2007b; Rahman et al., 2006). While the optimum range of temperature for *M. japonicus* growth is within 25-30°C (https://www.business.qld.gov.au/industry/fisheries/aquaculture). Taken all together, our results suggested that high temperature (33°C) reduced the spread of WSSV disease, but also had negative effect on the normal metabolism of shrimp, resulting in high mortalities in both WSSV-challenged and control shrimp groups.

In order to evaluate whether low temperature (15°C) affect WSSV pathogenicity, or, alternatively, WSSV was simply quiescent/inactive at the low temperature of 15°C, we monitored mortalities in shrimp challenged with WSSV and then transferred from 15°C to 25°C and from 25°C to15°C (Fig. 1, 3). We also determined the effect on WSSV replication at specific water temperature including 15°C, in terms of the presence of WSSV (Fig. 4A) as well as in terms of expression of WSSV transcripts (Fig. 4B). The finding that the delayed and reduced mortalities were observed when shrimp were transferred from 25°C to 15°C compared to shrimp held at 25°C before and after WSSV challenge, and that the increased mortalities were observed in shrimp shifted to 25°C when compared to mortalities of shrimp held continuously at 15°C (Fig. 3), indicating that low temperature (15°C) significantly influenced the pathogenicity of WSSV. Despite high survival (80%) and healthy shrimp appearance, incubation at 15°C did not result in elimination of WSSV. The low temperature of 15°C seemed to reduce rather than stop WSSV replication in infected shrimp. The viral accommodation concept, which implies that shrimp were not protected from infection, but rather protected from pathogenicity of the infecting virus, has been reported (Flegel, 2007). So, shrimp at 15°C

may act as carriers of WSSV and could spread the disease if the water temperature is increased.

Temperature can regulate the kinetics of virus replication, including absorption, synthesis of large molecules (e.g. protein and nucleic acid), enzyme activity and uncoating (Du et al., 2008; Ghosh and Bhattacharyya, 2007; Guan et al., 2003). Virus replication at 4°C does not proceed after virus attachment to the target cells because the cell membrane and cell metabolism are quiescent (Singh et al., 1995). Studies on binding of human T-lymphotropic virus type 1 (HTLV-1) virions showed that efficient binding required divalent calcium ions and temperatures higher than 20°C (Hague et al., 2003). Similarly, WSSV probably only attaches to the cell surface without or less replication at low temperature of 15°C. Indeed, the shrimp kept at 15°C ate less and were inactive as observed in our experiments. By amplifying *Wssv*VP28 and *Wssv*ORF332, which are classified as a major structural protein (van Hulten et al., 2001) and a latency-related gene (Dang et al., 2010), respectively, PCR analysis explored lower WSSV loads in surviving shrimp at 15°C when compared to that of shrimp maintained at higher temperatures (Fig. 4A). By measuring expression levels of WSSV transcripts (*Wssv*VP28, *Wssv*ORF332, *Wssv*ORF172, *Wssv*ORF188, *Wssv*ORF395, and *Wssv*ORF514) which are involved in the systemic infection of shrimp and viral DNA replication (Leu et al., 2009; van Hulten et al., 2001), RT-PCR analysis also explored less expression levels of these transcripts in surviving shrimp at 15°C when compared to that of shrimp maintained at higher temperatures (Fig. 4B). Taken together, PCR and RT-PCR data confirmed the mortality results of the effects of low temperature (15°C) on the pathogenicity of WSSV in kuruma shrimp. However, our hypotheses may need to be elucidated by additional bioassays.

4. CONCLUSIONS

In conclusion, our results indicated that the optimum temperature range for the growth of kuruma shrimp is also suitable conditions for replication of WSSV, and that kuruma shrimp was less susceptible at low temperature but may serve as a reservoir to spread the virus. The optimum temperature for replication of WSSV is around 25°C and at low temperature (e.g 15°C) kuruma shrimp may as a carrier carrying WSSV. These results could provide essential data for shrimp health management, in terms of considering the suitable season for culture and time period of culture, leading to the development of Better Management Practices (BMPs) for sustainable shrimp aquaculture.

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REFERENCES

Bui, T.M.D. (2010). On the epidemiology and evolution of white spot syndrome virus of shrimp. Ph.D thesis. Wageningen University.

Dang, L.T., Koyama, T., Shitara, A., Kondo, H., Aoki, T. and Hirono, I. (2010). Involvement of WSSV-shrimp homologs in WSSV infectivity in kuruma shrimp: Marsupenaeus japonicus. Antiviral Research, 88: 217-226.

de la Vega, E., Degnan, B.M., Hall, M.R. and Wilson, K.J. (2007a). Differential expression of immune-related genes and transposable elements in black tiger shrimp (*Penaeus monodon*) exposed to a range of environmental stressors. Fish and Shellfish Immunology, 23: 1072-1088.

de la Vega, E., Hall, M.R., Wilson, K.J., Reverter, A., Woods, R.G. and Degnan, B.M. (2007b). Stress-induced gene expression profiling in the black tiger shrimp *Penaeus monodon*. Physiological Genomics, 31: 126-138.

Du, H.H., Dai, W., Han, X., Li, W., Xu, Y. and Xu, Z. (2008). Effect of low water temperature on viral replication of white spot syndrome virus in *Procambarus clarkii*. Aquaculture, 277: 149-151.

Escobedo-Bonilla, C.M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M.B. and Nauwynck, H.J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. Journal of Fish Disease, 31: 1-18.

Flegel, T.W. (2007). Update on viral accommodation, a model for host-viral interaction in shrimp and other arthropods. Developmental & Comparative Immunology, 31: 217-231.

Guan, Y., Yu, Z. and Li, C. (2003). The effects of temperature on white spot syndrome infections in *Marsupenaeus japonicus*. Journal of Invertebrate Pathology, 83: 257-260.

Ghosh, S. and Bhattacharyya, S. (2007). Effect of temperature on viral infection and its control: a mathematical approach. Journal of Theoretical Biology, 247: 50-63.

Granja, C.B., Vidal, O.M., Parra, G. and Salazar, M. (2006). Hyperthermia reduces viral load of white spot syndrome virus in *Penaeus vannamei*. Diseases of Aquatic Organisms, 68: 175-180.

Hague, B.F., Zhao, T.M. and Kindt, T.J. (2003). Binding of HTLV-1 virions to T cells occurs by a temperature and calcium-dependent process and is blocked by certain type 2 adenosine receptor antagonists. Virus Research, 93: 31-39.

Jiravanichpaisal, P., Soderhall, K. and Soderhall, I. (2004). Effect of water temperature on the immune response and infectivity pattern of white spot syndrome virus (WSSV) in freshwater crayfish. Fish and Shellfish Immunology, 17: 265-275.

Jiravanichpaisal, P., Soderhall, K. and Soderhall, I. (2006). Characterization of white spot syndrome virus replication in in vitro-cultured haematopoietic stem cells of freshwater crayfish, *Pacifastacus leniusculus*. Journal of General Virology, 87: 847-854.

Johnson, K.N., van Hulten, M.C. and Barnes, A.C. (2008). "Vaccination" of shrimp against viral pathogens: phenomenology and underlying mechanisms. Vaccine, 26: 4885-4892.

Leu, J.H., Yang, F., Zhang, X., Xu, X., Kou, G.H. and Lo, C.F. (2009). Whispovirus. Current Topics in Microbiology and Immunology, 328: 197-227.

Lightner, D.V. and Redman, R.M. (1998). Shrimp diseases and current diagnostic methods. Aquaculture, 164: 201-220.

Mayo, M.A. (2002a). A summary of taxonomic changes recently approved by ICTV. Archives of Virology, 147: 1655-1663.

Mayo, M.A. (2002b). Virus taxonomy - Houston 2002. Archives of Virology, 147: 1071-1076.

Rahman, M.M., Escobedo-Bonilla, C.M., Corteel, M., Dantas-Lima, J.J., Wille, M., Alday-Sanz, V., Pensaert, M.B., Sorgeloos, P. and Nauwynck, H.J. (2006). 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*. Aquaculture, 261: 842-849.

Reyes, A., Salazar, M. and Granja, C. (2007). Temperature modifies gene expression in subcuticular epithelial cells of white spot syndrome virus-infected *Litopenaeus vannamei*. Developmental & Comparative Immunology, 31: 23-29.

Sanchez-Martinez, J.G., Aguirre-Guzman, G., and Mejia-Ruiz, H. (2007). White spot syndrome virus in cultured shrimp: A review. Aquaculture Research, 38: 1339-1354.

Singh, I.P., Chopra, A.K., Coppenhaver, D.H., Smith, E., Poast, J. and Baron, S. (1995). Vertebrate brains contain a broadly active antiviral substance. Antiviral Research, 27: 375-388.

van Hulten, M.C., Witteveldt, J., Snippe, M. and Vlak, J.M. (2001). White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. Virology, 285: 228-233.

Xu, H., Yan, F., Deng, X., Wang, J., Zou, T., Ma, X., Zhang, X. and Qi, Y. (2009). The interaction of white spot syndrome virus envelope protein VP28 with shrimp Hsc70 is specific and ATP-dependent. Fish and Shelfish Immunology, 26: 414-421.

https://www.business.qld.gov.au/industry/fisheries/aquaculture/aquaculture-species/kuruma-prawn-aquaculture/ cultural-environment-kuruma-prawns. Culture environment for kuruma prawns.