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Effect of temperature shifts on shrimp lightly infected with white spot syndrome virus (WSSV)

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ABSTRACT

Healthy juvenile kuruma shrimp, *Marsupenaeus japonicus*, and giant black tiger shrimp, *Penaeus monodon*, were immersed in a filtrate prepared from the epidermis of either white spot syndrome virus (WSSV)-infected *M. japonicus* or healthy *P. monodon* (control). Ten days after immersion, infection of the experimental shrimp were confirmed by two-step polymerase chain reaction (PCR) diagnosis. The sublethally infected and the healthy control shrimp were both subjected to different temperature conditions. For both shrimp species, the experimental groups began to die after 1 d of high temperature (29 ℃) treatment, while only a few shrimp died in the control groups. At low temperatures (15 ℃), the mortalities were lower in the first 24 h than when the shrimp were maintained at a water temperature of 24 ℃. With an initial exposure to low temperatures for 24 h, after the water temperature was increased to 24 or 29 ℃, *P. monodon* mortality reached 90%-100% after a further 36 h at either temperature, while at 29 ℃, *M. japonicus* mortality reached 100% within a further 48 h, but only reached 40% at 24 ℃. When WSSV DNA-specific primer sets were used for one-step and two-step diagnostic PCR, the moribund experimentally infected shrimp under temperature shifts all yielded the specific 1447-bp (WSSV DNA) PCR product in the first step. No 1447-bp PCR product was found in the control groups, whether experiencing a temperature shift or not. These results demonstrate that temperature shifts alone can induce an outbreak of WSS disease in populations in which viral infection is only two-step WSSV positive.

Key words: white spot syndrome, WSSV, *Penaeus monodon*, *Marsupenaeus japonicus*, polymerase chain reaction

INTRODUCTION

Outbreaks of virus-associated white spot syndrome (WSS) have led to serious mortalities among populations of cultured shrimp in different Asian countries (Inouye *et al.*, 1994; Momoyama *et al.*, 1994; Nakano *et al.*, 1994; Takahashi *et al.*, 1994; Cai *et al.*, 1995; Chou *et al.*, 1995; Wang *et al.*, 1995; Wongteerasupaya *et al.*, 1995; Lo *et al.*, 1996a; Peng *et al.*, 1998). A frequent characteristic of this disease is the presence of white spots on the exoskeleton and epidermis of the diseased shrimp, with the spots varying in size from barely visible to 3 mm in diameter. Histopathological studies have demonstrated that the causative agent, white spot syndrome virus (WSSV), most

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frequently attacks the cuticular epidermis, as evidenced by the presence in this tissue of degenerated cells characterized by hypertrophied nuclei (Wang *et al.*, 1995). The effects of WSSV pathogenesis are often acute, and frequently conclude with high mortality. Mortality typically reaches 90% within 2 to 7 d (Momoyama *et al.*, 1994; Chou *et al.*, 1995; Wang *et al.*, 1995). Recently, WSSV was reported to cause disease, high mortality rates, and latent infection in different species of shrimp, crab, and other arthopods (Lo *et al.*, 1996b, 1997).

Polymerase chain reaction (PCR) is a powerful diagnostic tool for the identification of pathogens (Erlich *et al.*, 1988), but the accurate identification of pathogens by PCR is dependent upon the availability of specific primers. As described previously, a WSSV genomic library has been constructed and specific primer sets have been designed and used for WSSV diagnosis (Wang *et al.*, 1995; Lo *et al.*, 1996a). WSSV in shrimp with obvious white spot syndrome is readily detectable by one-step PCR. Sensitivity can be increased (by a factor of about 10^3) by using nested primers in two-step PCR (Lo *et al.*, 1996a; Lo and Kou, 1998). Two-step PCR is able to detect the latent state of light WSSV infection in shrimp. A light WSSV infection is defined as occurring when shrimp with no clinical signs of white spots on the exoskeleton are WSSV one-step PCR negative but two-step PCR positive (Lo *et al.*, 1997). In this study, two-step PCR was therefore used to confirm that the experimentally infected shrimp had a light infection.

The factors affecting an individual shrimp's susceptibility to WSSV infection and the probability of its surviving such an infection are still unknown. In general, however, it is well known that water quality and temperature can affect the reproduction, growth, and survival of aquatic organisms (Chien, 1992). Thus, when cultured shrimp with a light WSSV infection were subjected to environmental stresses, the shrimp exhibited acute effects of WSSV infection with its typically high mortality rate (Chou *et al.*, 1995). In Taiwan, the production of cultured black tiger shrimp (*Penaeus monodon*) is a year-round operation, while cultured kuruma shrimp (*Marsupenaeus japonicus)* (formerly classified in the geneus Penaeus, Perez Farfante and Kensley, 1997) are grown mostly during the winter months (Chen, 1990; Liao, 1989). Temperatures in the ponds typically fluctuate annually between 12 °C and 30 °C (Chen, 1990). In this report, we investigate the effects of different temperatures and temperature shifts on outbreaks of WSS in lightly-infected *P. monodon* and *M. japonicus* under controlled conditions.

MATERIALS AND METHODS

Shrimp.

The juvenile *Penaeus monodon* and *Marsupenaeus japonicus* specimens used in this study were hatched and reared (to 0.2 g average weight) in the Tung Kang Marine Laboratory of the Taiwan Fisheries Research Institute. All of the shrimp were fed commercially produced shrimp food twice daily. All shrimp were apparently healthy with no clinical sign of WSS, and all sample specimens subjected to two-step WSSV diagnostic PCR were negative. The shrimp used for the challenge tests were inoculated at an ambient temperature of 22-24 °C, and then transferred to indoor tanks at water temperature of 24 °C for the temperature shift tests. Heating units were used to maintain water temperature.

Challenge test.

A filtrate containing the WSS virion was prepared by the method described by

Lo *et al.* (1996b). Briefly, exoskeletons (including attached epidermal tissue) to a total weight of approximately 1 g were removed from frozen, naturally diseased *M. japonicus* (Fig. 1) which were one-step WSSV-diagnostic PCR positive. These were extracted with 6 ml of cold 30-ppt sterilized seawater with vigorous vortexing. After being centrifuged at 3000 rpm (1700 xg) for 10 min, the supernatant was filtered though Whatman no. 3 filter paper and a 0.45-μm membrane (Sartorius). All the above procedures were carried out below 4 °C.

About 900 juvenile *P. monodon* and 900 juvenile *M. japonicus* (average weight 0.2 g) were separately cultured in six 3-l tanks at a density of 100 individuals/l. The filtrates in a series of 10-fold dilutions (10^0, 10^1 and 10^2 dilutions) were added to each tank in order to find a sublethal dose, with which we were able to obtain a two-step PCR-positive shrimp population with low mortality. The shrimp were bathed in the filtrates for 2 h and then transferred to six 67-l tanks containing 18 l of fresh seawater. Control populations were similarly prepared and exposed to the filtrate from healthy (i.e., two-step WSSV-diagnostic PCR negative) *P. monodon*. All shrimp were kept in the 67-l tanks for 10 d. Salinity was 30 ppt, and water temperature was maintained at 22-24 °C throughout this 10-d period.

Effects of temperature shifts.

Ten days after inoculation, randomly selected surviving juvenile *P. monodon* and *M. japonicus* of the 10^2 filtrate dilution shrimp group were transferred to sixteen 2-l tanks until each tank contained 10-12 individuals in 1.8 l of seawater. After being allowed to acclimate for 24 h, four temperature regimens were established (summarized in Table 1): In groups I and II, the shrimp were incubated either at a water temperature of 24 °C or 29 °C, respectively.

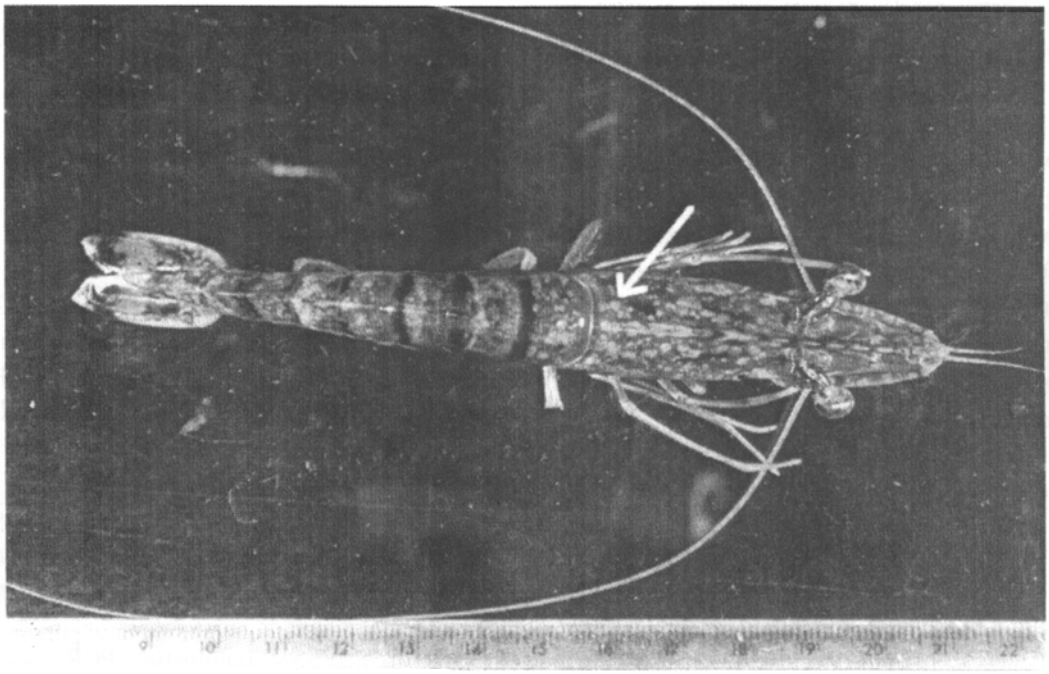

Figure 1. Diseased kuruma shrimp showing characteristic clinical signs of white spot syndrome (WSS). Arrow indicates obvious white spot.

Table 1. Summary of the various experimental designs on *Penaeus monodon* and *Marsupenaeus japonicus* with light WSSV infection.

Group I	24℃ (72h)	Experimental**	*P. monodon*
			M. japonicus
		Control*	*P. monodon*
			M. japonicus
Group II	29℃ (72h)	Experimental	*P. monodon*
			M. japonicus
		Control	*P. monodon*
			M. japonicus
Group III	15→24 ℃ (24h)(48h)	Experimental	*P. monodon*
			M. japonicus
		Control	*P. monodon*
			M. japonicus
Group IV	15→29℃ (24h)(48h)	Experimental	*P. monodon*
			M. japonicus
		Control	*P. monodon*
			M. japonicus

* Experimental populations were derived from only two-step PCR-positive shrimp populations which were bathed in the 10^2 dilution of WSSV filtrates for 2 h and then transferred to fresh seawater for 10 d.

** Control populations were similarly prepared but exposed to the filtrate from healthy (two-step WSSV-diagnostic PCR-negative) *P. monodon*.

Heating units were used to maintain the water temperature in insulated tanks. In groups III and IV, the shrimp in the beakers were incubated in a 15℃ incubator (Sony) for 24 h. The beakers were moved to insulated water and a water bath with heating units which were set at either 24℃ or 29 ℃. The water temperature was raised to either 24 ℃ or 29℃ within 2 h, and the shrimp were incubated in beakers for 48 h, respectively. One experimental and one control group for each species followed each regimen. Mortality was recorded twice daily, and moribund shrimp were collected every 12 h and subjected to WSSV-diagnostic PCR. After 72 h, all remaining shrimp whether moribund or not were similarly assessed.

Preparation of shrimp total DNA.

The isolation of template DNA for diagnostic PCR followed the method described by Lo *et al.* (1996a). Briefly, a recently excised shrimp head was placed in an Eppendorf tube containing 1 ml of digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulfate, 0.1 mg/ml proteinase K) and crushed with a disposable stick. After 1 h incubation at 65 °C, 0.6 ml of DNA supernatant was transferred to a new Eppendorf tube. After the NaCl concentration of the DNA solution was adjusted to 0.7 M by adding 100 µl 5 M NaCl, the solution was treated with 1% N-cetyl N,N,N-trimethylammonium bromide (CTAB) for 10 min at 65 °C. This was followed by successive extractions with an equal volume of chloroform/isoamyl alcohol (24:1) once, an equal volume of phenol (pH 7.9) two to three times, and two volumes of chloroform/isoamyl alcohol once. The DNA was recovered by ethanol precipitation, dried and resuspended in 0.1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at 65 °C for 30 min, and then stored at 4 °C.

WSSV-diagnostic PCR.

Oligonucleotide primers. The primers for one-step PCR consisted of 146F1 (5'-ACT ACT AAC TTC AGC CTA TCT AG-3') and 146R1 (5'-TAA TGC GGG TGT AAT GTT CTT ACG A-3'). The internal primer set for nested (two-step) PCR consisted of 146F2 (5'-GTA ACT GCC CCT TCC ATC TCC A-3') and 146R2 (5'-TAC GGC AGC TGC TGC ACC TTG T-3'). Both of these primer sets were originally developed by Lo *et al.* (1996a) and were synthesized on an Applied Biosystem synthesizer.

Before WSSV-diagnostic PCR was performed, the quality of the DNA extracted from moribund shrimp specimens

was checked by a primer set derived from the highly conserved region of decapod 18S rRNA (Kim and Abele, 1990; Lo *et al.*, 1996a): 143F (5'- TGC CTT ATC AGC TNT CGA TTG TAG-3', where N represents G, A, T or C) and 145R (5'-TTC AGN TTT GCA ACC ATA CTT CCC-3').

One-step WSSV-diagnostic PCR. Each 100-µl reaction volume contained 10 mM Tris-HCl, pH 8.8 at 25 °C, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 mM of each dNTP, 100 pmol 146F1/R1 primers, 2 units of DynaZyme™II DNA polymerase (Finnzymes Oy, Rihitontuntie 14 B, SF-02200 Espoo, Finland) and 1 µg of template DNA. The amplification was performed in an AG-9600 Thermal Station (Biotronics, Lowell, MA, USA). The temperature was first raised to 94 °C for 3 min, after which PCR amplification was performed over 40 cycles: 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min. After 40 cycles, an extension step at 72 °C for 5 min completed the PCR. Reaction mixtures containing no template DNA were run for all PCR reactions as negative controls. The final PCR products were checked by electrophoresis using 10 µl of aliquot on 1% agarose gel containing 0.5 µg/ml ethidium bromide. The results were visualized and photographed under an ultraviolet transilluminator.

Two-step WSSV-diagnostic PCR. After completion of the first step of PCR, 10 µl of the reaction mixture was added to 90 µl of a PCR cocktail containing the internal primer pair (146F2 and 146R2). This was subjected to a second series of 40 amplification cycles identical to those performed in the first step of amplification, and 10 µl of the reaction mixture was used for the analysis of PCR products.

RESULTS

Experimental infection for producing WSSV two-step PCR positive shrimp

Two-step WSSV PCR-positive shrimp were produced by immersing juvenile shrimp (*M. japonicus* and *P. monodon*) in a series of 10-fold dilution filtrates from diseased shrimp. Ten days after inoculation, the cumulative mortalities of shrimp groups that were immersed in 10^0 and 10^1 dilutions were very high, and random samples from these groups were mostly one-step PCR positive. The shrimp group immersed in the 10^2 dilution filtrate displayed low mortality, and three of the surviving juvenile *P. monodon* and *M. japonicus* were all one-step PCR negative and two-step PCR positive (Fig. 2). This was interpreted as confirming that a light infection had indeed been induced in the 10^2 dilution experimental shrimp. All control group specimens were two-step diagnostic PCR negative. The WSSV two-step PCR-positive shrimp and one-step PCR-negative shrimp were used for study in the subsequent experiments.

Effects of temperature on *Penaeus monodon* with light WSSV infection

Cumulative mortality in the *P. monodon* experimental group II (29 °C) reached 90% within 36 h (Fig. 3). In experimental group I (24 °C), the cumulative mortality reached only 50% after 36 h but 100% mortality by 72 h. No shrimp died in the control groups even at the higher temperature.

All surviving and moribund specimens in experimental groups I and II had become one-step PCR positive while the control groups with or without a temperature shift remained two-step PCR negative (Table 2, Fig. 4).

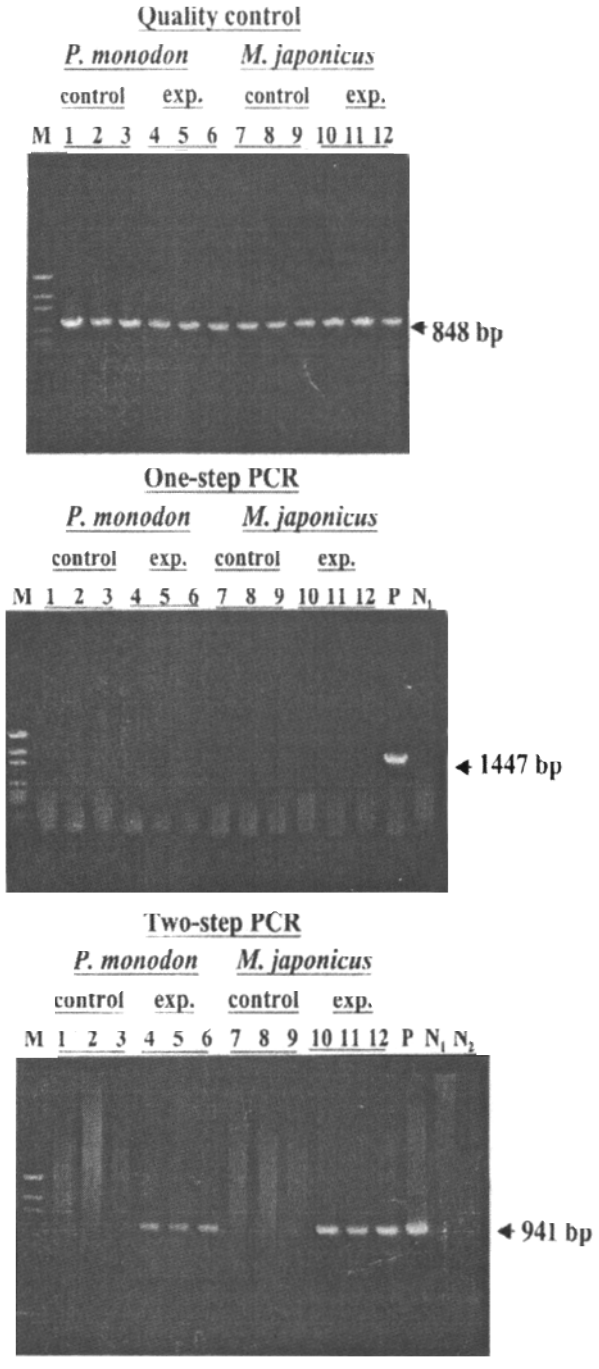

Figure 2. WSSV-specific DNA fragments amplified from template DNA samples prepared from surviving shrimp of *Penaeus monodon* and *Marsupenaeus japonicus* 10 d post-infection (by immersion in 10^2 dilution filtrate). Quality control: the quality of the extracted DNA was checked using the 143F/145R primer set which amplified the expected 848-bp (shrimp DNA) PCR product; one-step PCR: the 146F1/146R1 primer pair which amplified the expected 1447-bp (WSSV DNA) PCR product; two-step PCR: the 146F1/146R1 outer primer pair followed by the 146F2/146R2 inner primer pair which amplified the expected 941-bp (WSSV DNA) PCR product. M: pGEM DNA size marker; P: positive control. N1, N2: template-free control reaction initiated during each round of amplification.

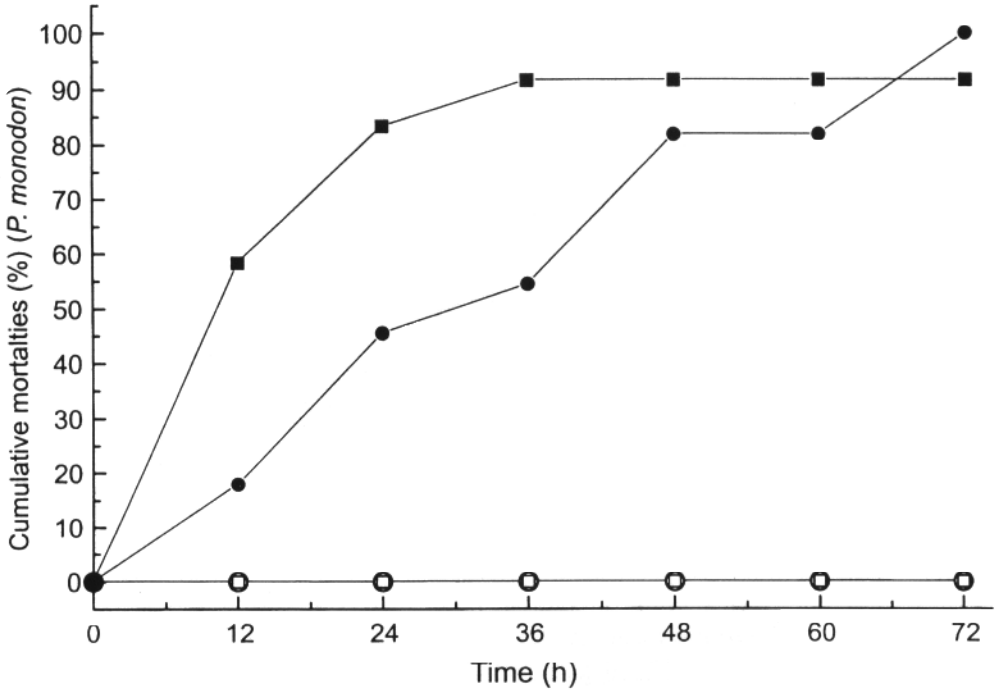

Figure 3. Cumulative mortality of lightly infected *P. monodon* under different temperature conditions. ○, Control group I; □, control group II; ●, experimental group I; ■, experimental group II.

Effects of temperature on *M. japonicus* with light WSSV infection

The *M. japonicus* experimental group II (29 °C) cumulative mortality reached 90% within 36 h (Fig. 5), whereas it reached only 10% within 36 h and 60% after 72 h in experimental group I (24 °C). In the control groups, the cumulative mortalities did not exceed 18% after 72 h, regardless of temperature shift.

All moribund specimens in experimental groups I and II were one-step PCR positive, while specimens from all the control groups whether surviving or moribund were all two-step PCR negative (Table 2, Fig. 6). Mortality in the control groups was thus shown not to be due to WSSV infection.

Effects of temperature shift on *P. monodon* with light WSSV infection

Cumulative mortalities were almost the same for both *P. monodon* experimental groups and control groups that were subjected to an initial 24 h of low temperature (15 °C) (Fig. 7). At the end of this first 24 h period, only 13%-30% mortality was observed. These mortality rates were lower than those for the first 24 h in the experimental groups held at 24 °C (45%) or subjected to a higher (29 °C) temperature (85%) (compare with Fig. 3). After the temperature was shifted up to 24 or 29 °C, however, the cumulative mortality rates increased reaching 80% and 100%, respectively. The temperature changes alone caused no mortality in the control groups.

All surviving and moribund specimens in experimental groups III and IV were one-step PCR positive, while those in the control groups were two-step PCR negative (Table 3, Fig. 8).

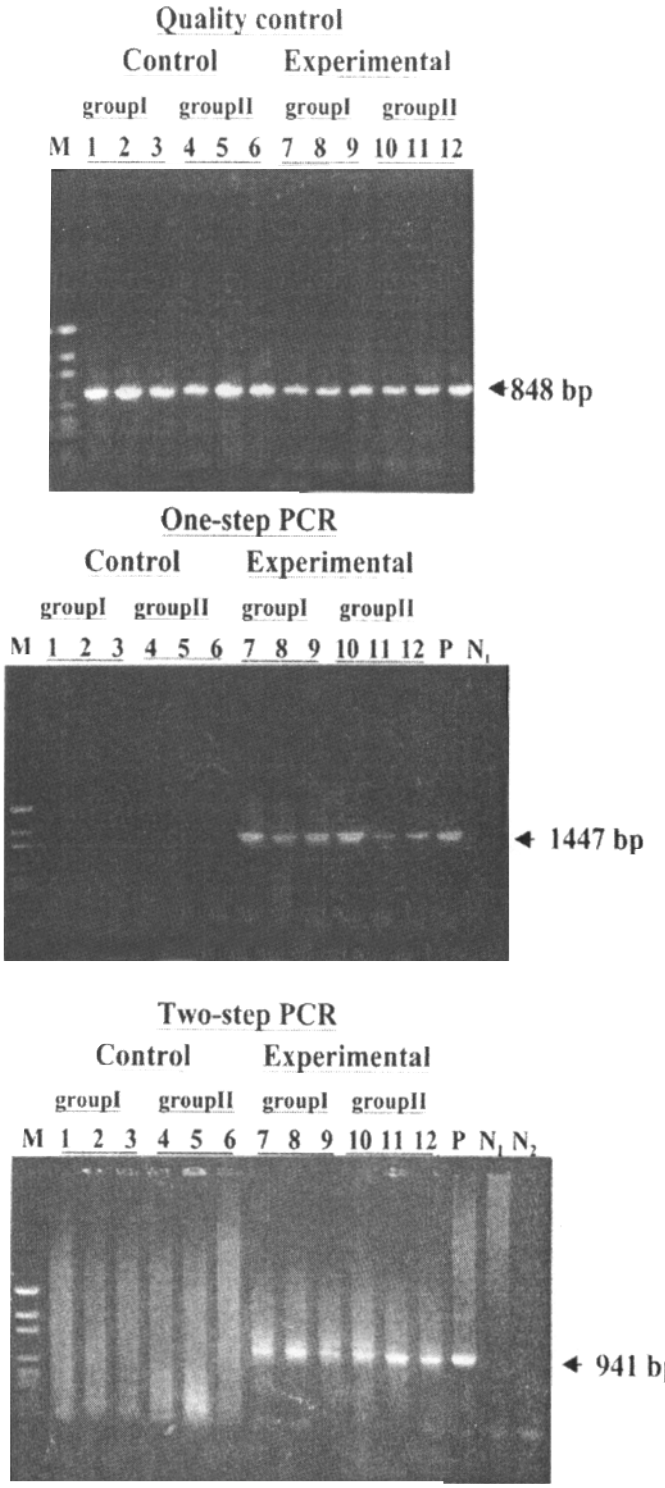

Figure 4. Effect of temperature on *P. monodon* by WSSV-diagnostic PCR. Template DNA was extracted from three of the surviving control and moribund experimental shrimp. Quality control, one- and two-step PCR, and size markers are detailed in Fig. 2.

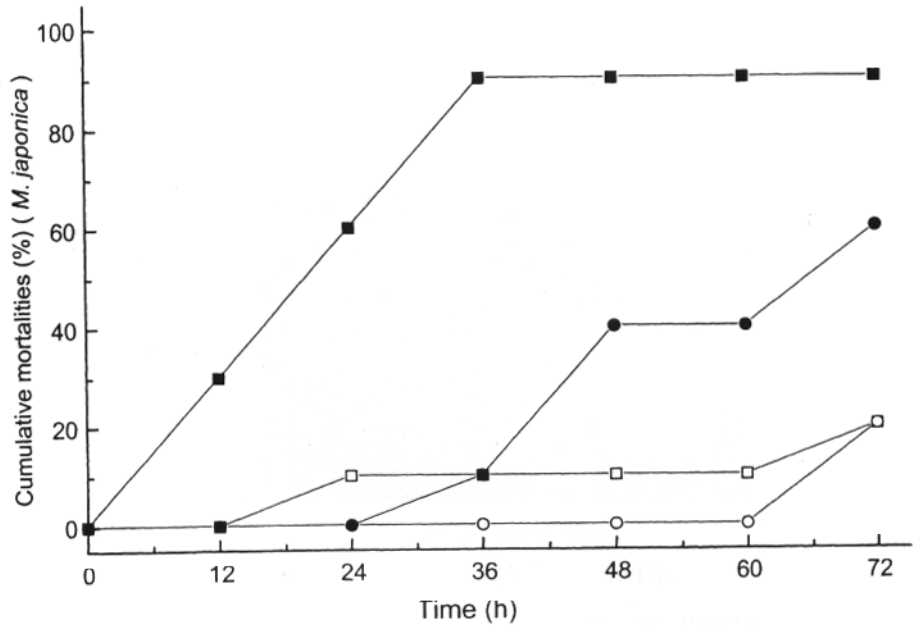

Figure 5. Cumulative mortality of lightly infected *M. japonicus* under different temperature condition. ○, Control group I; □, control group II; ●, experimental group I; ■, experimental group II.

Table 2. Summary of temperature effects on *P. monodon* and *M. japonicus* with light WSSV infection as determined by WSSV-diagnostic PCR.

WSSV-diagnostic PCR		*P. monodon*				*M. japonicus*			
		Control		Experimental		Control		Experimental	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Surviving	One-step PCR	0/11	0/11	0/0	1/1	0/10	0/11	2/4	0/1
	Two-step PCR	0/11	0/11	0/0	1/1	0/10	0/11	3/4	0/1
Moribund	One-step PCR	0/0	0/0	11/11	11/11	0/2	0/0	6/6	9/9
	Two-step PCR	0/0	0/0	11/11	11/11	0/2	0/0	6/6	9/9

Shrimp were incubated either at a water temperature of 24 or 29 ℃. Moribund shrimp were collected every 12 h and subjected to WSSV-diagnostic PCR. After 72 h, the surviving shrimp were also detected by WSSV-diagnostic PCR. Values represent the number of positive shrimp in the first or second step of PCR/number of shrimp examined.

Effects of temperature shift on *M. japonicus* with light WSSV infection

In the *M. japonicus* experimental groups under the initial low-temperature conditions, cumulative mortalities were very low (0%-10%) (Fig. 9). When the temperature was shifted up to 29 °C, however, the cumulative mortality reached 100% within a further 48 h. By contrast, only 40% mortality was observed when the temperature was shifted up to 24 °C. These significantly different mortality rates were similar to those already seen in Fig. 5. Not more than 10% mortality was found in the two control groups.

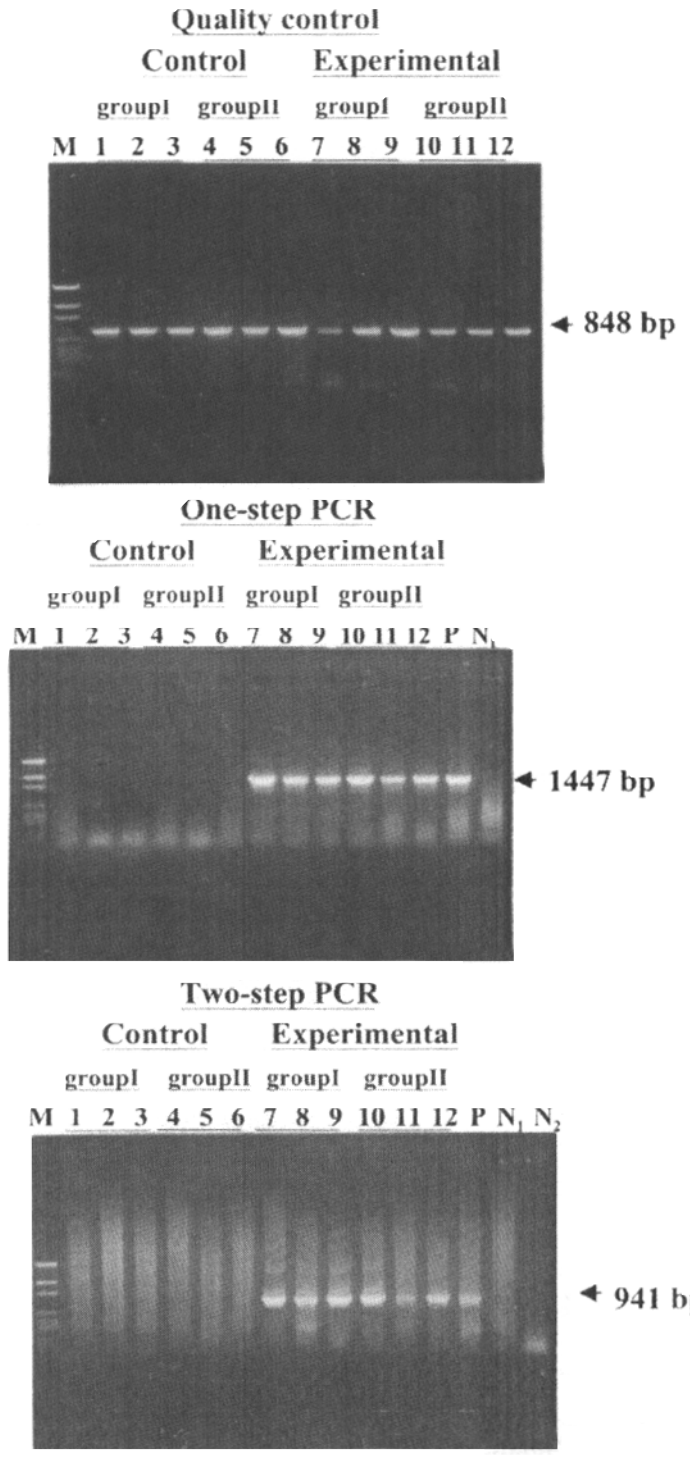

Figure 6. Effect of temperature on *M. japonicus* by WSSV-diagnostic PCR. Template DNA was extracted from three of the surviving control and moribund experimental shrimp. Quality control, one- and two-step PCR, and size markers are detailed in Fig. 2.

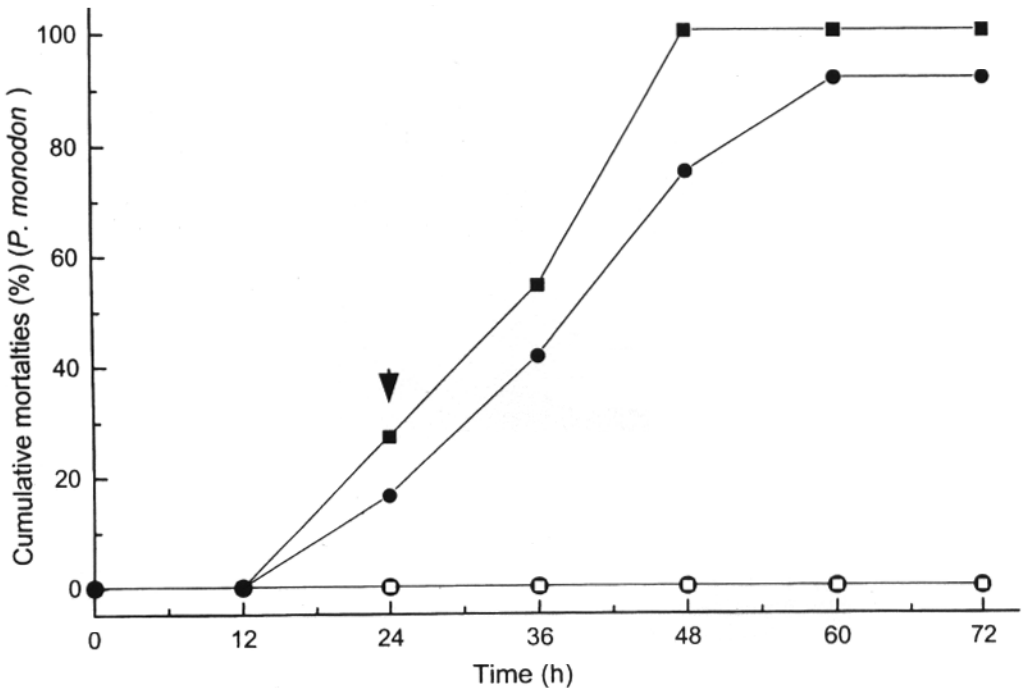

Figure 7. Effect on cumulative mortality of temperature shift from low temperature (15 ℃) to high temperature (29 ℃) on *P. monodon*. ○, Control group III; □, control group IV; ●, experimental group III; ■, experimental group IV. Arrow indicates the time at which the temperature was raised.

Table 3. Summary of effects of incubation at low temperature followed by shift to higher temperatures on *P. monodon* and *M. japonicus* with light WSSV infection as determined by WSSV-diagnostic PCR.

WSSV-diagnostic PCR		*P. monodon*				*M. japonicus*			
		Control		Experimental		Control		Experimental	
		Group III	Group IV	Group III	Group IV	Group III	Group IV	Group III	Group IV
Surviving	One-step PCR	0/11	0/10	1/1	0/0	0/11	0/10	7/7	1/1
	Two-step PCR	0/11	0/10	1/1	0/0	0/11	0/10	7/7	1/1
Moribund	One-step PCR	0/0	0/0	11/11	11/11	0/1	0/0	4/4	10/10
	Two-step PCR	0/0	0/0	11/11	11/11	0/1	0/0	4/4	10/10

Shrimp were incubated at 15 °C for 24 h then the temperature was raised to either 24 or 29 °C. Moribund shrimp were collected every 12 h and subjected to WSSV-diagnostic PCR. After 72 h, the surviving shrimp were also detected by WSSV-diagnostic PCR. Values represent the number of positive shrimp in the first or second step of PCR/number of shrimp examined.

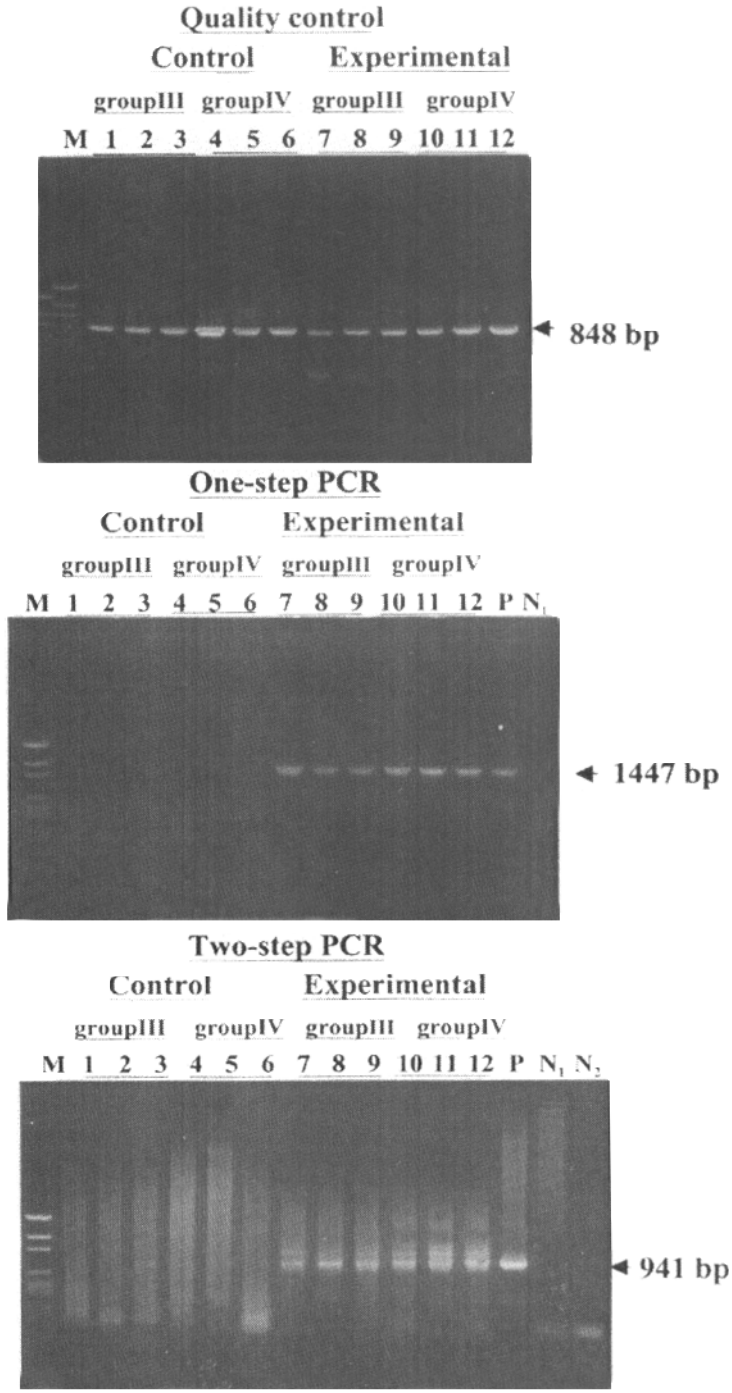

Figure 8. Effect of temperature shift from low to high temperature on *P. monodon* by WSSV-diagnostic PCR. Template DNA was extracted from three of the surviving control and moribund experimental shrimp. Quality control, one- and two-step PCR, and size markers are detailed in Fig. 2.

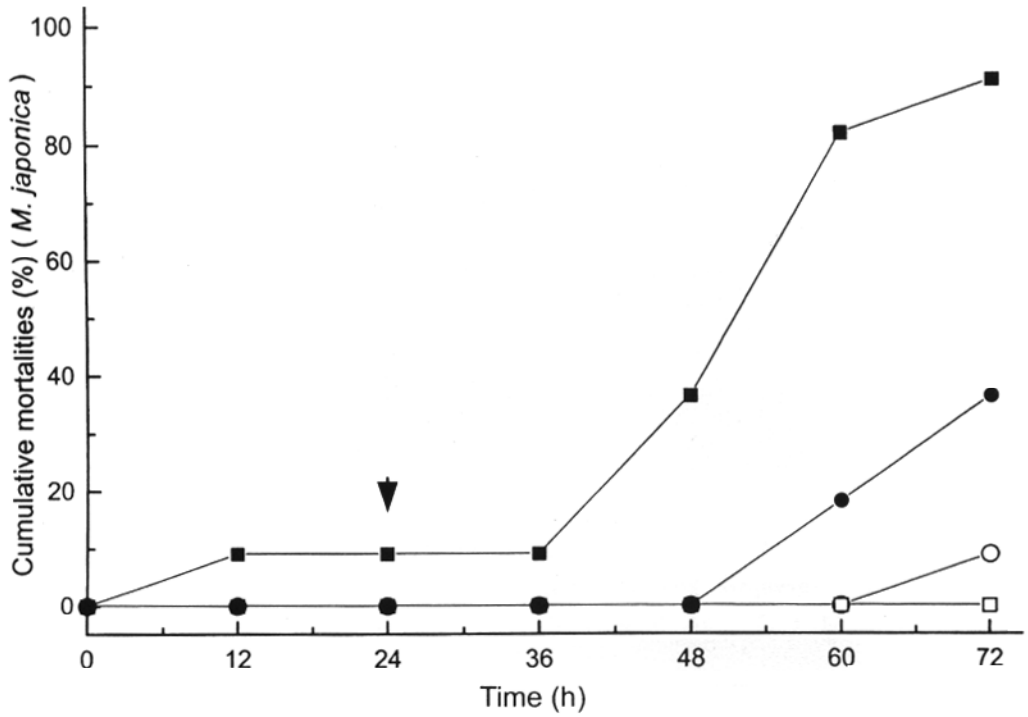

Figure 9. Effects on cumulative mortality of temperature shift from low temperature (15 ℃) to high temperature (29 ℃) on *M. japonicus.* ○, Control group III; □, control group IV; ●, experimental group III; ■, experimental group IV. The arrow indicates the time at which the temperature was raised.

All surviving or moribund specimens in experimental groups II and IV were one-step PCR positive, and the control groups were two-step PCR negative (Table 3, Fig. 10).

DISCUSSION

Under normal conditions, most cultured marine shrimp are eurythermal. Temperature is one of the most important factors controlling the growth of shrimp. Tolerance to variations in temperature often differs among closely related species due to genetic differences (Fast and Lester, 1992). The most suitable temperature range for *P. monodon* culture is 25-30 °C and they cease feeding below 18 °C (Liao, 1989). Although *M. japonicus* is considered to be a temperate species, it can withstand a wider temperature range of from 15 to 33 °C. The optimal temperature for *M. japonicus* is 25 °C. It can survive under 15 °C but stops feeding, growth, and molting, and at 12 °C even ceases swimming and jumping (Korringa, 1976; Chen, 1990). A temperature of 15 °C would therefore have been stressful, but 29 °C would have been within the preferred temperature range of both species. According to our present data, however, shifts from a lower temperature to higher temperatures hastened outbreaks of WSS disease in both lightly infected *P. monodon* and *M. japonicus*. The reason may be that WSSV lightly infected shrimp become, or are already, weaker, and are more susceptible to viral infection and replication. Stress either lowers the resistance of the host or enhances the pathogenicity of the pathogens. The mortality of WSSV lightly infected *M. japonicus* was more pronounced

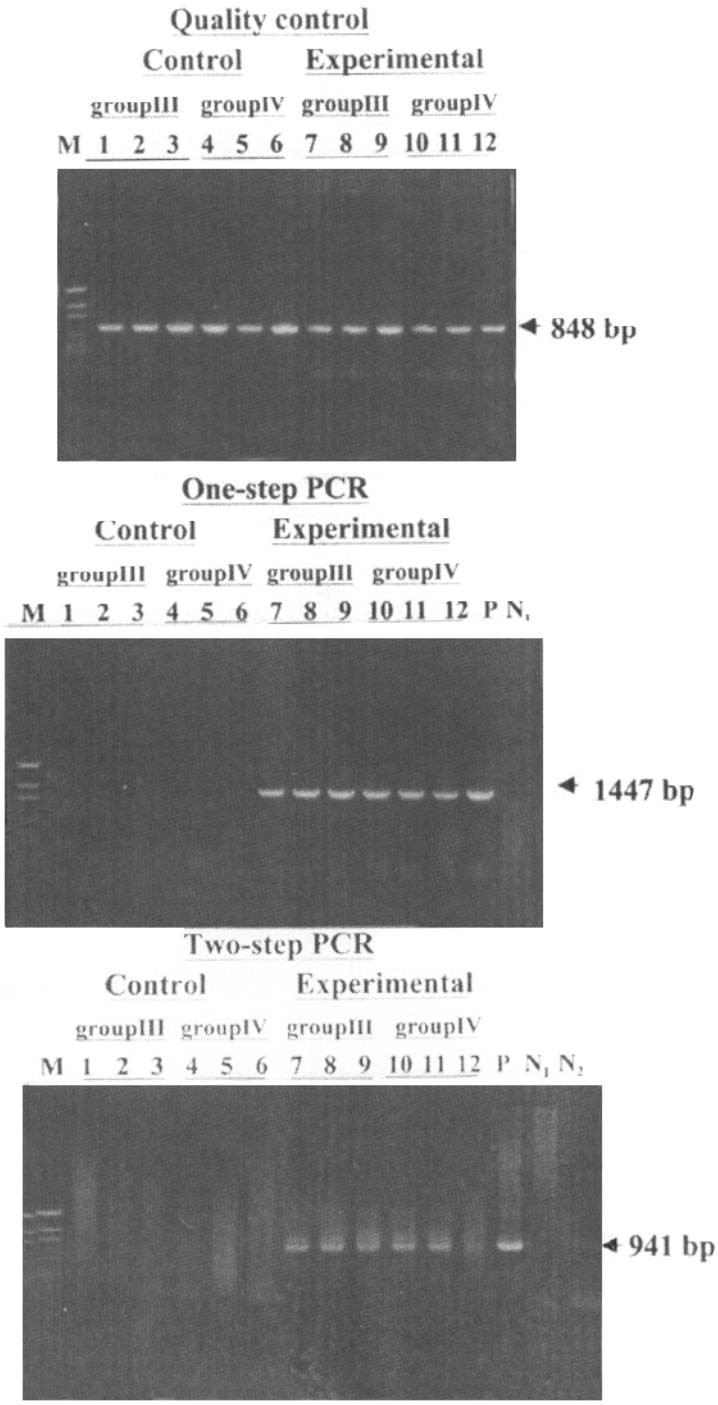

Figure 10. Effect of temperature shift from low to high temperature on M. japonicus by WSSV-diagnostic PCR. Template DNA was extracted from three of the surviving control and moribund experimental shrimp. Quality control, one- and two-step PCR, and size markers are detailed in Fig. 2.

when the culture temperature was shifted from 15 °C to 29 °C (Fig. 9). However, when WSSV was used to inoculate shrimp at 30 °C, which is a typical temperature for culture pond water in southern Taiwan in the summer, it did not always lead to high mortality rates or outbreaks of WSS disease. It should be emphasized that there is a difference between dramatic environmental changes and slower long-term change: the former is a source of stress while the latter leads to adaptation. Under conditions prevailing in the wild, shrimp may gradually adapt to rising temperatures and maintain a stable physiological condition (Blundon, 1989). Thus high temperature alone is not sufficient to induce WSS disease outbreak. In contrast, a dramatic shift to a higher temperature may be a harmful, although not in and of itself a lethal stress on shrimp. Thus, the rapidly accumulated mortality of WSSV lightly infected shrimp at high temperature was mainly due to the rate of temperature shift, and not the high temperature itself.

The mechanism by which a temperature increase affects outbreaks of this viral disease is still unknown, but a disruption or disturbance of physiological stability is presumably involved. The ability to buffer stress is important in lessening damage from dramatic environmental changes, and this ability may vary across species. Thus, as reported here, at least at postlarval or juvenile stages, *P. monodon* was more sensitive to a temperature increase and was more susceptible to WSSV than was *M. japonicus*, although the source of WSSV was derived from diseased *M. japonicus* (compare Figs. 7, 9). Furthermore, the mortalities of *P. monodon* were broadly similar at a water temperature of 24 °C and at a higher temperature (29 °C) after 72 h (Figs. 3, 7); while this same temperature difference of only 5 °C produced differences in the experimental *M. japonicus* groups, with those at room temperature exhibiting a much lower mortality (Figs. 5, 9). These phenomena may reflect the different physiologies of the two respective shrimp species.

At lethal low temperatures, metabolic processes slow down below the level required for cellular maintenance. In *P. aztecus* postlarvae, the length and weight increased more rapidly at higher temperatures (32 and 25 °C) than at low temperatures (18 and 11 °C) regardless of salinity conditions (Zein-Eldin and Aldrich, 1965). At a low temperature (15 °C), the metabolic rates of WSSV lightly infected shrimp were low, and the WSSV replication rate decreased. So the mortalities of WSSV lightly infected *M. japonicus* and *P. monodon* were low at this low temperature. All moribund shrimp after high-temperature treatment were one-step PCR positive, which clearly suggests that WSSV is the main pathogen. Although the pathogenicity of WSSV can be altered by several factors such as culture conditions, host conditions, and life-stage, the data from this study imply that temperature shift alone can be a sufficient cause for a WSS disease outbreak to occur in a lightly infected population. This might be particularly critical in the case of brood shrimp. Up to now, seed production has been totally dependent on the capture of spawning brood shrimp, but over 40% of wild-caught shrimp used as brooders have a light WSSV infection (Lo *et al.*, 1996b; Hsu *et al.*, 1999). In the absence of a reliable and economical screening protocol, particular care should therefore be taken to minimize potentially stressful environmental changes, especially shifts in temperature.

At low temperature, *M. japonicus* shows reduced metabolism (Mauro and Mangum, 1982; Blundon, 1989; Pasterson, 1993) and respiration rate. Oxygen uptake can be interrupted at low temperature by a

failure of the oxygen transport pigment in the blood to release oxygen in the cold (Mauro and Mangum, 1982). At low temperatures, nerve and muscle function in crustaceans becomes physically blocked (Blundon, 1989). Respiration rates have also been published for *P. monodon* (Liao and Murai, 1986; Kurmaly *et al.*, 1989), and these differ from the rates reported for *M. japonicus* (Mauro and Mangum, 1982; Blundon, 1989; Pasterson, 1993). These differences in respiration rates may reflect corresponding metabolic differences and may thus explain the differences observed between these two species in the present study.

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溫度改變對白點症病毒潛伏感染蝦類之影響

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摘　要

蝦類白點症病毒是目前嚴重危害台灣及其他地區養殖蝦類的重要病原體。本研究乃利用罹病死亡之斑節蝦表皮及蝦殼配製病毒液，以不同稀釋度浸泡健康之草蝦及斑節蝦，以造成蝦類潛伏性感染，再施以不同溫度的緊迫，觀察蝦體的死亡狀況。在高溫緊迫的環境下，潛伏感染草蝦及斑節蝦於一天內即迅速死亡，對照組則無。溫度由 15℃提高至 24℃時，於 72 小時內潛伏感染草蝦的死亡率即達 100%，而潛伏感染斑節蝦的死亡率僅約 40%；當溫度由 15℃提高至 29℃時，不論潛伏感染草蝦或斑節蝦其死亡率均高達 100%，顯示感染病毒的草蝦對於高溫緊迫因子的適應性比感染病毒的斑節蝦低。利用聚合酶鏈反應證明其均因白點症桿狀病毒感染而死亡。本研究結果顯示溫度變化造成的緊迫的確會引爆白點症的發生。

關鍵詞：白點症、白點症病毒、草蝦、斑節蝦、聚合酶鏈反應