

Effect of white spot syndrome virus (WSSV) and water exchange on survival and production of *Litopenaeus vannamei* under semi-intensive culture conditions.

El efecto virus de la mancha blanca (WSSV) y el recambio de agua sobre la supervivencia y producción de *Litopenaeus vannamei* bajo condiciones de un cultivo semi-intensivo.

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ABSTRACT

A study was performed in two commercial shrimp farms in the province of Guasave, north of Sinaloa, Mexico, to assess the effects of the presence of the white spot syndrome virus (WSSV) and of water exchange on the growth rate, production, and survival of the Pacific white shrimp, *Litopenaeus vannamei*, during the fall-winter season in semi-intensive culture ponds. The experiment consisted of four treatments; in the first (T1), three earthen ponds with water exchange, were stocked with PCR-positive for white-spot syndrome virus (WSSV) postlarvae. In the second (T2), three earthen ponds were stocked likewise (PCR-positive), but without water exchange. In the third (T3), three earthen ponds, with water exchange were stocked with PCR-negative for white-spot syndrome virus (WSSV) postlarvae. In the fourth (T4), three earthen ponds were stocked likewise (PCR-negative), but without water exchange. The average growth rates were 0.56, 0.56, 0.80, and 0.75 g/week for T1, T2, T3, and T4, respectively. Survival was 23.2% (T1), 26.1% (T2), 64.3% (T3), and 66.1% (T4). Production ranged between 252.60 and 847.00 kg/ha, with the lowest production in T2 and the highest in T3. Feed conversion ratio ranged from 1.00 for T3 to 1.70 for T2. The final average weight ranged between 10.6 g (T2) to 12.5 g (T3). The WSSV can affect negatively the growth rate (30%), the survival (64%), and the production (69%) in comparison with PCR-negative organisms. No differences in weight were found between WSSV-infected and non-infected individual shrimps, as well as in nested-PCR positive against single-step PCR positive organisms. It found that the zero water exchange strategy could be feasible for the culture of the white shrimp *L. vannamei* at a commercial level during the fall-winter season.

Key words: Water exchange, white spot syndrome virus, shrimp culture.

RESUMEN

El presente estudio se llevó a cabo en dos granjas comerciales, localizadas en el Municipio de Guasave, en el norte del Estado de Sinaloa, México, para estimar los efectos del virus del síndrome de mancha blanca (WSSV) y del recambio de agua sobre la tasa de crecimiento, producción y supervivencia del camarón blanco del Pacífico, *Litopenaeus vannamei*, durante la estación templada (otoño-invierno) en un cultivo semi-intensivo. El experimento consistió de cuatro tratamientos; en el primero (T1), tres estanques rústicos con recambio de agua fueron sembrados con postlarvas PCR-positivas para el síndrome del virus de la mancha blanca. En el segundo (T2), tres estanques rústicos fueron sembrados (postlarvas PCR-positivas), pero sin recambio de agua. En el tercero (T3) tres estanques rústicos con recambio de agua fueron sembrados con postlarvas PCR-negativas para el síndrome del virus de la mancha blanca. En el cuarto (T4), tres estanques rústicos fueron sembrados con postlarvas (PCR-negativas), pero sin recambio de agua. La tasa de crecimiento media fue de 0.56, 0.56, 0.80 y 0.75 g/semana, para los tratamientos 1, 2, 3 y 4 respectivamente. La supervivencia fue de 23.2% (T1), 26.1% (T2), 64.3% (T3), y 66.1% (T4). El intervalo de producción fue de 252.60 a 847.00 kg/ha, obteniendo la menor producción en el T2 y la más alta en el T3. La tasa de conversión de alimento presentó un intervalo de 1.00 en T3 a 1.70 en T2. El peso promedio final fue de 10.6 g (T2) a 12.5 g (T3). El WSSV puede afectar negativamente la tasa de crecimiento (30%), la supervivencia (64%) y la producción (69%), en comparación con organismos que son PCR-negativos. No se encontraron diferencias en el peso entre los camarones infectados y no infectados, ni en camarones determinados positivos en el PCR anidado en comparación con los determinados positivos en el PCR simple. Se encontró que la estrategia de cero recambio de agua puede ser factible para el cultivo del camarón blanco *L. vannamei* a escala comercial durante la estación de otoño-invierno.

Palabras clave: Recambio de agua, virus mancha blanca, cultivo de camarón.

INTRODUCTION

In Mexico, shrimp farming has been developed mainly in the Northwest region, in the states of Sonora, Sinaloa, and Nayarit. Environmental conditions have allowed the culture of *Litopenaeus vannamei* Bonne, 1931 only during a limited time period, when the water temperature of the culture pond is above 24°C. These conditions are present from mid-March to November, a period when *L. vannamei* presents an average growth rate of 0.98 g/week. During the remainder of the year, water temperature is below 20°C, which represents a limiting factor for shrimp growth (Ponce-Palafox *et al.*, 1997).

The economic impacts caused by viral diseases during the last few years, the increasing environmental pollution induced by the effluents of shrimp farms, and the abundance of shrimp during July-August and November-December, with the consequent decrease in prices, have brought low profitability for this activity. Farmers need alternative strategies for both reducing the risk of infection and obtaining a better selling price for their product. The use of species tolerant to low temperatures, such as *Penaeus chinensis* (Osbeck, 1765) (Lee & Wickins, 1992) has been considered, but production of exotic species is not recommended, as they may become the access route of other pathogens (Lightner, 1988; Lightner, 1994).

Another alternative developed by some farmers is seeding the shrimp at the beginning of autumn (October) to harvest in

March-April when shrimp is sold at the best price. Apparently the results have been satisfactory but have not been disseminated as this activity is mostly restricted to the private sector, where much of the information is kept among them. However, it has been determined that stocking at temperatures below 20°C induces stress in postlarvae making them susceptible to WSSV, for this reason it has been considered not advisable to stock postlarvae during the cold months (winter).

On the other hand, most of the shrimp farms in Mexico manage a water exchange rate between 10 and 15% of the total volume of the ponds per day, which represents about 10% of the operational costs. However, recent studies have demonstrated that the water exchange rate can be reduced without negative effects on production, both at the pre-rearing (Cohen *et al.*, 2001) and the rearing stages (Hopkins *et al.*, 1995; Moss *et al.*, 2001), and it has been demonstrated that high production rates can be obtained in systems without water exchange (Browdy, 2001). Hence, it is important to assess, at the commercial level, a strategy of zero water exchange under different health conditions of the shrimp.

The present research was aimed at studying the culture of the white shrimp *L. vannamei* during the colder months (October-March) in a commercial system with and without water exchange and under the influence of the white spot syndrome virus (WSSV) to determine whether production parameters are suitable to sustain a commercial culture.

MATERIALS AND METHODS

Study area. The study was performed for 90 days, in two commercial farms located in the north of Sinaloa, Mexico (25°17'20" LN, 108°28'57" LW; 25°17'59" LN, 108° 29'58" LW), during the fall-winter period of 2001-2002. Postlarvae for pond stocking were obtained from two different hatcheries. In the first farm, shrimp were naturally infected, being PCR-positive (T1 and T2) for the white-spot syndrome virus (WSSV), and in the second farm shrimp were PCR-negative (T3 and T4) for the virus. In both cases, two strategies were used, one with water exchange and the other without water exchange.

Experimental procedures. The experimental design was randomized with three replicates per treatment. The experimental units consisted of 12 earthen ponds of 5,000 ± 300 m² per treatment. Water was pumped from the Boca del Rio estuary and discharged into the ponds by means of a deriving channel. For T1 and T3, 15 to 20% of the water was exchanged daily, whereas for T2 and T4 only the water lost through evaporation or filtration was replenished.

Water quality. The following water quality parameters were determined for each pond: temperature, dissolved oxygen concentration, salinity (determined with a YSI 85), pH (potentiometer), nitrites, nitrates, and reactive phosphorus, according to Arredondo-Figueroa & Ponce-Palafox (1998). Temperature and dissolved oxygen were recorded daily at 6:00 and 13:00 h, salinity and pH at 13.00 h. Nitrites, nitrates, and reactive phosphorus were determined twice a month. Measurements were made at the floodgate of each pond.

At the end of the experiment, the ponds were drained and shrimp were harvested at the output gate. A sample of 200 organisms was taken from each pond to determine the average final weight and survival.

Collection of samples. Fifty postlarvae (0.012 g) and fifty juvenile (3-5 g and 10-11 g) shrimps showing clinical signs of white-spot syndrome (mass mortalities 45 days, reddish body, erratic swim) were collected from grow-out ponds from the first farm. Healthy animals were collected from the second farm in the same way. Shrimps were transported in an ice chest to the CIIDIR-Sinaloa, for analysis. They were stored at -70 °C until processed. Also, postlarvae from farm 2 were analyzed by collecting 150 shrimp per batch. Postlarvae were transported alive to the laboratory in large plastic bags with seawater, and DNA was immediately extracted.

Analysis of the samples. Each sample of 50 shrimps was grouped in subsamples of 10 organisms. Lamellar tissue from every shrimp of each subsample was pooled in a single tube for DNA extraction. A nested PCR analysis was done per group. After the quick removal of lamellar tissue, frozen shrimp were returned to storage at -70 °C. DNA from the 10 shrimps of each subsample

that gave a positive PCR result was individually extracted and PCR-analyzed to determine the number of individuals that were PCR-positive from each of these groups. PCR-negative groups were not reanalyzed. Postlarvae were organized in groups of 20 PLs per DNA extraction.

Viral and shrimp DNA co-extraction. Shrimp lamellar tissue (50–100 mg) was used for each extraction. For DNA extraction, DNAzol reagent (Invitrogen, Carlsbad, CA, USA) was used following manufacturer's instructions. DNA samples were quantified spectrophotometrically at 260 nm.

PCR. DNA was amplified by polymerase chain reaction (PCR) using the published primers and methods for WSSV (Peinado-Guevara & Lopez-Meyer, 2006). The expected size of the amplified fragment was 982 bp for the first PCR reaction and 570 bp for the nested PCR reaction. The PCR reaction mixture for singlestep and nested PCR reactions included 0.4 mM dNTP, 4 mM MgCl₂, 0.5 AM WSSV primers, 1.25 U Taq DNA polymerase (Promega, Wisconsin, USA) and 1 X Taq buffer, provided by the enzyme manufacturer. The volume of the reaction was 25 µl. In single-step PCR reactions, 2–3 ng of target DNA were used. For nested PCR, 1 µl of a 1:100 dilution of the single-step PCR reaction was used as target DNA. Amplification was performed in a DNA thermocycler iCycler (Biorad, Hercules, CA, USA) using the following parameters: initial denaturation at 95°C for 4 min, followed by 40 cycles of 95°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. Following this, aliquots of the PCR reactions were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, visualized under UV light, and then photographed.

Growth and Feed. *L. vannamei* postlarvae (P₁₂) were seeded in each pond at 10 organisms/m². During the experiment, shrimps were fed with "camaronina" 35, a commercial feed with 35% of protein content manufactured by Purina (4% lipids, 5% fiber, 12% moisture). Shrimps were fed 10% of their biomass during the first two weeks of the study, followed by 3% of the biomass for the remainder of the study. A sample of 100 shrimps per pond was taken out weekly to determine average weight and to adjust the feeding rate.

Statistical analysis. Since each experiment was performed in different but neighboring farms, statistical analyses were performed according to Montgomery (1984) applying a Student's t test and factorial design with two factors. To assess statistically significant differences between treatments, Tukey's test was applied at a probability level of $p = 0.05$.

RESULTS

Water quality. Water quality conditions in the ponds during the study presented a wide range of fluctuations: water temperature

ranged from 13.0 to 29.5°C at 6:00 h and from 15.6 to 31.6°C at 13:00 h; dissolved oxygen concentration was 5.4 mg/L at 6:00 h, 8.0 mg/L at 13:00 h, with concentrations above 3.5 mg/L most of the time; salinity varied from 28 to 42‰. Nitrites, nitrates, and reactive phosphorus concentrations, as well as the pH, of the ponds with or without water exchange are shown in table 1. Intervals of water quality parameters were within acceptable levels in both shrimp farms (Arredondo-Figueroa & Ponce-Palafox, 1998)

Significant differences ($\rho=0.05$) between the ponds with and without water exchange. were only found for the dissolved oxygen concentration measured at 6:00 and 13:00 h, with the highest values for ponds with water exchange.

Detection of WSSV in cultured shrimp by PCR. Shrimp were collected from various ponds on the two farms. These animals showed no signs of WSSV infection and were apparently healthy (Fig. 1, lanes 1-8) and WSSV was detected in only one farm (Fig. 2, lanes 1-10).

WSSV and Growth. Regarding biometric parameters and production yield, no significant differences were found between the ponds with and without water exchange (Table 2). The effect of WSSV was significant on the growth rate, survival, and produc-

tion. The final weight of the shrimps ranged from 10.6 to 12.5 g, finding smaller sizes in the ponds with WSSV-positive organisms. Survival rates were higher in the ponds with WSSV-negative organisms (64.3% to 66.1%) as compared to the WSSV-positive ponds (23.2% to 26.1%), with higher survival rates in the ponds without water exchange. The Feed Conversion Ratio (FCR) revealed no significant differences between treatments, although lower FCRs were found in the WSSV-negative ponds (1.0 to 1.3) with water exchange, and higher in the WSSV-positive ponds (1.5 to 1.7) without water exchange. Growth rates (0.75 to 0.80 g/week) and the final production (793.2 to 847.0 kg/ha) were significantly higher ($\rho = 0.05$) in the WSSV-negative ponds.

DISCUSSION

The differences in water quality in the ponds with and without water exchange in semi-intensive and intensive systems, are similar with those found by Allan and Maguire (1993), and demonstrate that water exchange has no significant effect on the pH and the concentration of nitrites and nitrates; only a decrease in phosphorus concentration occurs in ponds with water exchange. It has been determined that the ponds without

Table 1. Average of physico-chemical parameters of the water in the ponds with and without water exchange.

Parameters	With water exchange	Without water exchange
Temperature 06:00 h (°C)	20.0 ± 8.3 ^a	19.9 ± 7.5 ^a
Temperature 13:00 h (°C)	22.9 ± 8.0 ^a	22.7 ± 7.9 ^a
Dissolved Oxygen 06:00 h (mg/L)	5.4 ± 3.8 ^a	5.4 ± 3.3 ^a
Dissolved Oxygen 13:00 h (mg/L)	7.8 ± 3.4 ^a	8.0 ± 3.9 ^a
Salinity	35.1 ± 6.5 ^a	35.4 ± 7.0 ^a
pH	8.2 ± 0.7 ^a	8.2 ± 0.6 ^a
Nitrites (mg/L)	0.02 ± 0.03 ^a	0.01 ± 0.02 ^a
Nitrates (mg/L)	0.09 ± 0.12 ^a	0.09 ± 0.18 ^a
Reactive phosphorus (mg/L)	0.23 ± 0.38 ^a	0.30 ± 0.57 ^b

Different superscripts indicate significant differences ($\pi=0.05$).

Table 2. Growth, survival, production, and feed conversion ratio (FCR) in shrimp ponds with and without water exchange.

Parameter/Treatments	WSSV-positive		WSSV-negative	
	T1	T2	T3	T4
Pond area	5,000 ± 100a	4,800 ± 200a	5,100 ± 150a	5,150 ± 200a
Initial weight (g)	0.013 ± 0.010a	0.011 ± 0.013a	0.013 ± 0.009a	0.012 ± 0.011a
Final weight (g)	11.9 ± 2.8a	10.6 ± 2.2a	12.5 ± 0.9a	12.0 ± 1.5a
Growth rate (g/week)	0.56 ± 0.15b	0.56 ± 0.13b	0.80 ± 0.02a	0.75 ± 0.01a
Survival (%)	23.2 ± 2.9b	26.1 ± 1.6b	64.3 ± 0.6a	66.1 ± 1.2a
Production (kg/ha)	274.6 ± 33.6b	252.6 ± 18.2b	847.0 ± 28.8a	793.2 ± 34.3a
FCR	1.5 ± 0.2a	1.7 ± 0.6a	1.0 ± 0.1a	1.3 ± 0.3a

Different superscripts indicate significant differences ($\rho=0.05$).

T1 = With water exchange, WSSV-positive; T2 = Without water exchange, WSSV-positive; T3= With water exchange, WSSV-negative; T4 = Without water exchange, WSSV-negative.

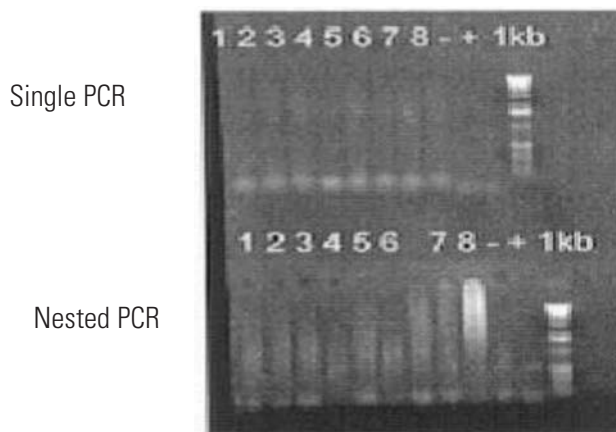


Figure 1. PCR-negative detection of white spot syndrome virus DNA in shrimp collected from ponds. Lanes 1-8 represent a negative *Litopenaeus vannamei*. Lane end molecular marker (1-kb ladder).

water exchange present a higher organic load and biochemical oxygen demand (Hopkins *et al.*, 1996). Critical oxygen concentrations of 0.6 mg/L were recorded in the morning in the ponds with water exchange, whereas in the ponds without water exchange, minimum oxygen concentration was 1.8 mg/l, revealing a better condition in the latter.

No significant differences were found in the final weight, growth rate, production, and survival between the ponds with and without water exchange, although a slight tendency to smaller sizes and lower yields was found in ponds without exchange of water, a result previously reported by Allan and Maguire (1993) and Hopkins *et al.* (1996) in intensive systems. A 7% higher production was found in the ponds with water exchange than in those without water exchange, a similar relation has been found in intensive systems (Hopkins *et al.*, 1996). In the USA, it has been

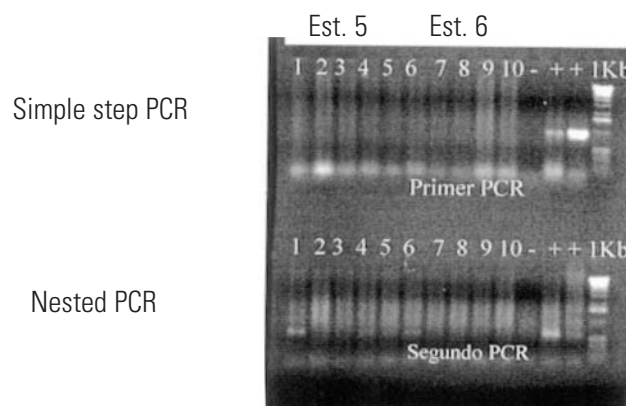


Figure 2. PCR-positive detection of white spot syndrome virus DNA in shrimp collected from ponds. Lanes 1-10 represent a positive *Litopenaeus vannamei*. Lane end molecular marker (1-kb ladder)

reported that the costs of energy in intensive shrimp culture with water exchange are 31.5% higher than without water exchange (Hopkins *et al.*, 1996); while in the northwest of Mexico the energy costs are 15% higher in the semi-intensive systems with water exchange than without water exchange.

The growth rate of shrimps in disease-free ponds during the warm season (28 to 30°C) ranges from 0.9 to 1.8 g/week (Clifford, 1985); whereas in the colder season (13 to 22°C) it is around 0.6 to 0.8 g/week. During the fall-winter season, the growth rate decreased to 0.56 g/week due to the effect of WSSV, 20% lower than the growth rate observed in WSSV-negative organisms. Nevertheless, these shrimp can reach the commercial size of 10.0 to 12.0 g in five months (November-March).

It was not surprising to find that shrimp in the sample were positive for WSSV by PCR without gross or histological signs of disease. The ability of shrimp to carry WSSV for relatively long periods under good rearing conditions has been previously reported (Tsai *et al.*, 1999; Withyachumnarnkul, 1999; Khadijah *et al.*, 2003). The main effect of WSSV is on the survival rate (Lawrence *et al.*, 2001), as it may fluctuate from 8% to 26% in semi-intensive commercial systems, as observed in this work. If rapid growth during early viral infection is a real phenomenon, understanding the physiological mechanism could lead to practical management or nutritional strategies for accelerated shrimp growth. Although survival of WSSV-positive organisms was low at the end of the experiment, the culture was profitable, because prices of shrimp in this season are high.

The recorded production level with water exchange treatments in WSSV-positive organisms (252.60 to 274.60 kg/ha) was lower than the yields reported for this species at warmer temperatures (24 to 31°C), both at experimental (Browdy *et al.*, 1993; Martínez-Cordova *et al.*, 1998) and commercial levels (800 to 2,000 kg/ha). The recorded yield in WSSV-negative organisms (847 kg/ha) was acceptable.

In conclusion no significant differences were observed in water quality or production yields in the ponds with or without water exchange. The growth rate in the fall-winter season is 30% lower than in the warm season, and the yield in WSSV-infected ponds was 69% lower than in disease-free ponds.

The WSSV can affect negatively the growth rate (30%), the survival (64%), and the production (69%), in comparison with PCR-negative organisms.

No differences in weight were found between WSSV-infected and non-infected individual shrimps, as well as in nested-PCR positive against single-step PCR positive organisms.

Based on these results, and considering the existing infrastructure (more than 30,000 ha) in the Northwest of Mexico, we

conclude that the zero water exchange strategy might be feasible for the commercial culture of the white shrimp *L. vannamei* a low density level during the fall-winter season (October- March).

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