Detection of white spot syndrome virus on *Penaeus monodon* and *Metapenaeus dalli* from Bulacan, Philippines

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Shrimp aquaculture is an integral source of revenue in developing tropical countries of Southeast Asia such as the Philippines. In the early 1990s the country ranked third in the shrimp international market, however the industry slid back to the 13th as it is presently plagued with outbreaks of diseases which caused massive decrease in production. Among these diseases, the white spot syndrome virus (WSSV) remains one of the most potent and infectious for most known species of cultivated penaeid shrimps. In this light, the presence of the WSSV in *P. monodon* and *M. dalli* both collected from the same sampling site were investigated. Polymerase chain reaction (PCR) utilizing specific primers were used to detect WSSV infection in shrimp hemolymph. Results showed 20% WSSV prevalence from three sampling sites in *P. monodon* while none was detected in all *M. dalli* samples. These results, coupled with the fact that both species were collected from the same pond, suggest that *M. dalli* is more resilient to WSSV compared to *P. monodon*. Moreover, it points out that domesticated shrimp such as, *P. monodon* is more susceptible than incidental shrimps like the *M. dalli*. This interesting discovery will have impact in the current shrimp culture management and selective breeding practices of the country.

**Keywords:** *Metapenaeus dalli*, *Penaeus monodon*, white spot syndrome virus, polymerase chain reaction, virus detection

INTRODUCTION

Inflicting diseases on today’s aquaculture wreaked havoc on the sustainable growth of shrimp culture industry worldwide. In the Asia-Pacific countries, the industry suggests annual losses of about 4 billion US dollars [1]. In the inter-tropical countries, shrimp is an indispensable source of revenue, as this commodity accounts for almost 20% of aquaculture commodities in trade [2]. In the Philippines, the infiltration of these diseases resulted into a downgrade from the third most prolific shrimp industry worldwide during the 1990’s to being only the 13th at present. A large fraction of the damage to the industry is undoubtedly...
caused by viruses, which already accounted to have over 20 strains affecting both penaeid shrimp wild stocks and commercial production [3].

Among the known virus affecting shrimp culture, the white spot syndrome virus (WSSV) is known to be the most potent and widespread pathogen. This virus can spread rapidly, and can cause massive mortality in a span of 3–10 days post-infection [4, 5]. Transmission of the virus does not solely depend on shrimps but also on other crustaceans, which can be migrating reservoirs [6]. The insufficient modes of sanitation, the growing number of cultures all over the country coupled with uncontrolled trade movement account for the optimum conditions for the virus to spread to almost all farming areas.

Although there are already some methods which displayed efficacy against the virus under experimental conditions, no effective treatments are available to address WSSV infection in the field [7]. One of the promising solutions proposed is the establishment of specific pathogen free (SPF) shrimp culture [8]. Among the conditions for establishing such broodstock is to have an available source of wild shrimps that are genetically diverse and free from any pathogen [9, 10]. To date, there is yet no cost-effective treatment for WSSV [11]. Thus, prevention is still the best way to avoid outbreaks. In the prevention of disease outbreaks on shrimp cultures, prescreening of the culture on the presence of any pathogen is necessary [12]. There is a dearth of information on the detection of WSSV in several farming areas in the country. The work of Magbanua et al., (2000) was the latest and only report covering the region [16]. Hence, this study aims to detect the presence of WSSV from two species of shrimp collected in Hagonoy, Bulacan. It is noteworthy to mention, that this is the first detection of WSSV that includes M. dalli as target species in the entire country.

**EXPERIMENTAL**

**Sampling.** Samples of P. monodon and M. dalli, were collected from three private ponds in San Pablo Hagonoy, Bulacan, Philippines. Five shrimps of both species were obtained from each pond, summing up to thirty shrimp samples for the whole study. From each sample, the hemolymph was extracted on site and was stored separately in microcentrifuge tubes with 0.5 mL sodium citrate in DEPC-treated water. The rest of the samples were kept in ice and transported to the Biochemistry/Molecular Biology Laboratory at the Thomas Aquinas Research Complex (TARC) of the University of Santo Tomas for storage and processing. Three samples of each species were also brought to the National Fisheries of Research and Development Institute, Bureau of Fisheries and Aquatic Resources for proper identification.

**DNA isolation.** The shrimp samples were dissected obtaining the gills, heart and hepatopancreas tissue samples. Samples were then placed separately in labeled microcentrifuge tubes containing 0.5 mL Tripure isolation reagent (see Table 1 for labelling) and was homogenized. Genomic DNA was extracted following Roche Tripure Isolation Reagent (Roche, U.S.A.) protocol and recommendations. The isolated DNA was then quantified using UV Spectrophotometry.

**Polymerase chain reaction.** Polymerase chain reaction (PCR) was conducted using WSSV primer [13], LMT primer [14] and (EF-1α) elongation factor-1α [15] as standard control (primer sequence shown in Table 1. Thermocycler conditions consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for
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30 sec, 55°C for 30 sec, 72°C for 30 sec and final extension at 72°C for 5 min. PCR products were then visualized in 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Detection of WSSV has become a routine measure to ensure quality of fry, juveniles and adult shrimps being cultured. In the Philippines however, only one study in Bulacan Province which has several hectares of shrimp farming area, has been reported [16]. This study was conducted to detect the presence of WSSV in two cultured shrimp species collected in Hagonoy, Bulacan.

The randomly collected samples of *P. monodon* and *M. dalli* did not show any gross signs of WSSV infection (i.e., white spots on the carapace, etc.). However, absence of external manifestations is not conclusive that there is an absence of WSSV infection, and it may just be that the virus is present but may not be potent enough, such that the host immune system can still carry out some resistance [16–18]. Nonetheless, any shrimp positive for WSSV is a threat as it can serve as a reservoir of the virus which facilitates viral transmission [6,19]. WSSV transmission can happen via shrimp-shrimp (horizontal) and likewise parent-larvae (vertical) [20].

This clearly points out that WSSV infection screening through mere physical examination is insufficient and inaccurate. Moreover, the mere appearance of white spots is an unreliable criterion for diagnosis as white spots can also be produced by other environmental conditions or organisms such as bacterial infections which closely resemble the white spots on the cuticle produced by WSSV in the absence of WSSV infection. Therefore, screening for WSSV through molecular methods plays an important role in the shrimp culture quality assessment. Among other molecular mode of diagnosis, PCR-based detection is the most suited for WSSV detection [21, 22].

Detection of WSSV on the three sampling sites yielded a consistent (1/5) 20% WSSV prevalence among *P. monodon*, compared to a study conducted by Magbanua et al. (2000) which likewise involved five *P. monodon* shrimp in the same province but yielded (3/5) 60% WSSV infection. The inconsistency of these results is not unusual but points out that the intensity of the infection of WSSV depend on numerous factors such as, temperature, water salinity, pH, etc. [23]. It is also in the early 2000, that the country’s shrimp production has been gravely affected by WSSV infection. On the other hand, samples of *M. dalli* from the three sampling sites were all uninfected, and thus gave 0% WSSV prevalence (0/5). Nested-PCR, a more sensitive than single-step PCR [4, 24], was also conducted to double check the single PCR negative result. Results of the nested PCR corroborated the findings of the single step PCR results (data not shown).

### Table 1. Primers used for PCR amplification

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<tr>
<th>Primer Name</th>
<th>Oligonucleotide Sequence</th>
<th>Source</th>
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| LMT-F       | 5' GAAACTATTGAAAAGGCTTTCCCT 3'  
               | 5' GTTCCTTATTATATCTACTACGGCAA 3' | Tapay et al., 1999 [14] |
| LMT-R       | 5' GTACGGCAATACTGGAGGAGG 3'  
               | 5' GGAGATGTTGTAAGATGGATAG 3' | Takashi et al., 1996 [13] |
| WSSV-F      | 5' ATGGTTGTTGCAACTTTGCCCC 3'  
               | 5' TTGACCTCCTTGATCACACC 3' | Maningas et al., 2008 [15] |
| WSSV-R      | 5' ATGGTTGTTGCAACTTTGCCCC 3'  
               | 5' TTGACCTCCTTGATCACACC 3' | Maningas et al., 2008 [15] |

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The striking difference of WSSV prevalence between *P. monodon* and *M. dalli* shrimp is intriguing as samples were collected from the same pond. Several risk factors or stressors which arise from physiochemical properties of water and pond bottom: the fluctuation of pH, temperature, and salinity may affect viral potency [1]. Temperature alone for instance influence metabolism, oxygen consumption, feeding rate, growth, molting, survival and tolerance to toxic metabolites [25, 26]. Aside from the species difference of *P. monodon* and *M. dalli*, an interesting fact is that, those infected came from domesticated broodstocks. On the other hand, the uninfected *M. dalli* shrimps are coming from the wild that incidentally enters the pond with the body of water. The domestication confines the shrimps in a very conducive environment for growth and development, making them more vulnerable to pathogens in the long run. As for the *M. dalli*, shrimp were all tested negative for WSSV, these maybe because these shrimps are just migratory shrimps that were incidentally present on the same pond, and therefore can be referred to as mere incidentals. These incidental shrimps are more resilient than farmed shrimp, and this can be accounted for by the fact that these shrimps are coming from the wild and have been exposed to a lot of stressors and are therefore more rigid than the domesticated *P. monodon*.
Taken all together, our findings that domesticated *P. monodon* are more susceptible to WSSV than the *M. dalli* which are just incidentals will have implication in shrimp farm management and selective breeding practices of the country.

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REFERENCE


