Assessment of the Roles of Copepod *Apocyclops royi* and Bivalve Mollusk *Meretrix lusoria* in White Spot Syndrome Virus Transmission

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**Abstract** Here, we investigate the roles of copepods and bivalve mollusks in the transmission of white spot syndrome virus (WSSV), which is the causative pathogen of an acute, contagious disease that causes severe mortalities in cultured shrimp. Copepods are common components in seawater ponds and are often eaten as live food by shrimp post-larvae. WSSV has been detected in these animals, but it is unknown whether this was due to contamination or infection. Meanwhile, the bivalve mollusk *Meretrix lusoria* is often used as live food for brooders, and in Taiwan, this hard clam is sometimes co-cultured with shrimp in farming ponds. However, mollusks’ ability to accumulate, or allow the replication of, shrimp viruses has not previously been studied. In this study, WSSV, the copepod *Apocyclops royi* and bivalve mollusk *M. lusoria* were experimentally challenged with WSSV and then assayed for both the presence of the virus and for viral gene expression. Results showed that the WSSV genome could be detected and that the viral loads were increased in a time-dependent manner after challenge both in *A. royi* and *M. lusoria*. Reverse transcriptase PCR monitoring of WSSV gene expression showed that WSSV could replicate in *A. royi* but not in *M. lusoria*, which suggested that WSSV, while could infect *A. royi*, was only accumulated in *M. lusoria*. A bioassay further showed that the WSSV accumulated in *M. lusoria* could be transmitted to *Litopenaeus vannamei* and cause severe infection.

**Keywords** WSSV · Copepods · Mollusks · *Apocyclops royi* · *Meretrix lusoria*

**Introduction**

White spot disease (WSD) is an acute disease of shrimp that has caused severe mortalities and huge economic losses to the shrimp farming industry globally for more than a decade (Lo et al. 1996b, 2005; Escobedo-Bonilla et al. 2008). This disease was first reported from an outbreak of cultured penaeid shrimp that occurred in northeast Asia in 1993 (Nakano et al. 1994; Takahashi et al. 1994). White spot syndrome virus (WSSV) is the extremely virulent causative agent of this disease. WSSV is a large, enveloped double-stranded DNA virus of approximately 275×120 nm in size with an olivaceous to bacilliform shape (Wang et al. 1995). This virus was assigned to the genus *Whispovirus*, family Nimaviridae (Vlak et al. 2004). WSSV has a wide host range that extends not only to most shrimp species but also to many other crustaceans, including crayfish, crab, and lobster (Huang et al. 1995; Lo et al. 1996a; Peng et al. 1998; Wang et al. 1998; He et al. 1999; Otta et al. 1999). The virus’ wide host range has contributed to its wide
geographic distribution, while its ability to infect a range of non-cultured species has allowed the virus to bypass control measures designed to prevent the spread of the disease in shrimp farms.

In aquatic animal health surveillance/control programs, all of the susceptible species in the relevant ecosystem should be considered. This includes live feed organisms. These animals play an important role in the dietary regimen of shrimp, and as a general rule, they are cultured in farming ponds before shrimp post-larvae are stocked. Copepods are common components in seawater ponds and are often eaten as live food by shrimp post-larvae during the first month of rearing after stocking in the pond (Reymond and Lagardère 1990). However, it has been demonstrated that copepods can act as mechanical vectors for bacterial pathogens (Huq and Colwell 1996), and demonstrated that copepods can act as mechanical vectors for viral pathogens (Meyers 1984; Mortensen et al. 1990; Starliper et al. 1998; Lees 2000; Perez Farfante and Kensley 1997), were bought from National Taiwan Ocean University Aquatic Animal Center. All these experimental animals were checked with nested WSSV diagnostic PCR (see below) and confirmed to be WSSV-free. They were kept in 25 ppt seawater with continuous aeration.

Materials and Methods

Experimental Animals

The A. royi copepods used in this study were laboratory-reared mono-cultured animals. Samples of the clam M. lusoria were bought from a local market. Samples of the other experimental animal, Litopenaeus vannamei (Perez Farfante and Kensley 1997), were bought from National Taiwan Ocean University Aquatic Animal Center. All these experimental animals were checked with nested WSSV diagnostic PCR (see below) and confirmed to be WSSV-free. They were kept in 25 ppt seawater with continuous aeration.

WSSV Inoculum Preparation

The virus used in this study, the WSSV-TW strain (Chang et al. 2008), originates from a batch of WSSV-infected Penaeus monodon collected in Taiwan in 1994 (Wang et al. 1995; Lo et al. 1999). The WSSV inoculum was prepared as described previously (Tsai et al. 1999). Briefly, frozen specimens of the gills and exoskeleton from the original batch of WSSV-infected P. monodon were minced and then homogenized in 4°C pre-chilled 0.9% NaCl (0.1 g ml⁻¹). After centrifugation at 800× g for 10 min at 4°C, the supernatant was diluted 1:100 with 0.9% NaCl and filtered through a 0.45-μm filter. For experimental infection, 50 μl of the filtrate was injected into each adult specific pathogen-free L. vannamei at the dorsal lateral area of the fourth abdominal segment. Three days after injection, hemolymph was collected from the moribund L. vannamei and the hemocytes were removed by centrifugation at 1,000× g for 10 min at 4°C and filtration through 0.45-μm filters. The supernatant was then diluted with a double volume of sterile phosphate-buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄) and stored at −80°C as virus stock.

Infectivity of WSSV on A. royi

For the WSSV infectivity studies on A. royi, the virus stock described previously was 10× diluted with cold PBS and filtered through a 0.45-μm filter. The diluents were further diluted 50× with seawater containing the mono-cultured alga Isochrysis galbana (bought from Tungkang Biotechnology Research Center, Taiwan Fisheries Research Institute) to a final volume of 50 ml. The final suspension was therefore a 500× dilution of the original virus stock. A. royi were then challenged with WSSV by immersion in this virus inoculum for 3 h. During the challenge, aeration was sufficient to give good oxygenation and keep the WSSV in suspension. After challenge, A. royi were transferred to
250 ml of fresh seawater containing *I. galbana*. At 0 h (i.e., before challenge), 3 h (i.e., at the end of the challenge period), 24, 48, and 72 h post-challenge (hpc), samples of the copepods were collected, rinsed with a large amount of sterilized seawater, and subjected to RT-PCR, nested WSSV diagnostic PCR, and real-time PCR.

**Infectivity of WSSV on *M. lusoria***

For WSSV infectivity studies on *M. lusoria*, the WSSV stock was diluted 10× with cold PBS and filtered through a 0.45-μm filter. Samples of the diluted WSSV inoculum were further diluted using seawater with *I. galbana* to make suspensions that were 500×, 1,000×, 2,000×, and 4,000× dilutions of the original virus stock. *M. lusoria* were challenged with WSSV by immersion using these different suspensions. At 0, 3, 24, 48, and 72 hpc, *M. lusoria* were sampled, rinsed with a large amount of sterilized seawater, and their gills collected for RT-PCR, nested WSSV diagnostic PCR, real-time PCR, and a bioassay experiment.

**Template DNA Preparation for Nested WSSV Diagnostic PCR and Real-Time PCR**

A DNA extraction kit (Farming IntelligenGene Tech) consisting of “DTAB solution,” “CTAB solution,” and “dissolving solution” was used to prepare template DNA from samples of pooled *A. royi*, gills of *M. lusoria*, and pleopods of *L. vannamei*. The DNA extraction protocol followed the instruction manual provided with the kit, and the final DNA pellet was resuspended in double-distilled water. For *M. lusoria* and *L. vannamei*, DNA was adjusted to the standard concentration (Lo et al. 1996b), but because much less extracted DNA was available from *A. royi*, a much lower concentration was used for this species.

**Nested WSSV Diagnostic PCR**

Nested primer sets (consisting of the outer primer pair, 146F1/146R1, and the nested primer pair, 146F2/146R2) were used for nested WSSV diagnostic PCR (Lo et al. 1996b). Using this method, samples that only tested positive in the second (nested) step of PCR were classified as “lightly infected,” while samples that were positive in the first step were classified as “heavily infected.” In the challenge experiment with *M. lusoria*, the presence of WSSV was detected only by using the first step of nested WSSV diagnostic PCR.

In the original method (Lo et al. 1996b), the quality of the DNA extracted from the tested animals was checked with a primer pair (143F/145R) that amplified a decapod gene fragment before the PCR amplification of WSSV genomic DNA. However, the *A. royi* and *M. lusoria* genomic DNA failed to be amplified by 143F/145R. Therefore, in order to successfully amplify the genomic DNA of all three species, the primers 18SF1 and 18SR1 were designed from the highly conserved regions of the 18S ribosomal RNA (rRNA) gene nucleotide sequences of *A. royi*, *Meretrix meretrix*, and *L. vannamei* (*M. meretrix* was used because the selected conserved region of *M. lusoria* 18S rRNA gene sequence was not publicly available). The 18SF1/18SR1 primers were based on published sequences with GenBank accession nos. AF186250, EF426291, and AF186250, respectively (Fig. 1). The *M. lusoria* 18S rRNA gene fragment was then successfully amplified by the primer pair 18SF1/18SR1, and the amplified fragment was also confirmed by sequencing. Sequences of the 18SF1 and 18SR1 amplified *M. lusoria* 18S rRNA gene fragment are shown in Fig. 2. The amplicon size of the 18S rRNA gene fragments amplified by the 18SF1 and 18SR1 in *A. royi*, *M. lusoria*, and *L. vannamei* are 631, 637, and 651 bp, respectively. The sequences of all the primers used here are shown in Table 1. The thermal cycling program and reaction conditions for PCR were the same as those described previously (Lo et al. 1996b), except that amplification of the 18S rRNA gene fragments was run for 30 cycles only.

**Temporal Transcription Analysis of WSSV Gene Expression by RT-PCR**

Total RNA was extracted from pooled samples of *A. royi* and gills of *M. lusoria* harvested at each time point, purified with TRIzol Reagent (Invitrogen Corp.), and then treated with DNase I (Invitrogen Corp.) to remove any residual DNA. The RNAs were reverse-transcribed with SuperScript III reverse transcriptase (Invitrogen Corp.) and an oligo(dT) anchor primer (Roche). The first-strand cDNA products were subjected to PCR with the indicated primers (Table 2). For monitoring WSSV gene expression profiles in the challenged *A. royi* and *M. lusoria*, the WSSV *ie1* (an immediate early gene), *dnapol* (a DNA polymerase gene), *vp664* (a nucleocapsid protein gene), and *vp28* (an envelope gene) were chosen as targets. Primers and PCR conditions for these targets are shown in Table 2. All PCR products were confirmed by sequencing.

**Fig. 1** Part of an alignment constructed from selected gene sequences of *A. royi*, *M. meretrix*, and *L. vannamei* 18S rRNA. Shaded regions show the highly conserved domains used to design the primers 18SF1 and 18SR1 in this study. GenBank accession numbers are given for each sequence. Alignments of amino acid sequences were made in CLUSTAL_X (Thompson et al. 1997)
protein gene) gene fragments were all amplified from the same templates using the primer pairs ie1-F/ie1-R, dnapol-F/dnapol-R, 419-F/419-R, and vp28-F/vp28-R, respectively (Table 2). An 18S rRNA gene primer pair, 18SF1/18SR1, was used as an internal control for RNA quantity and amplification efficiency. To confirm that there was no WSSV DNA contamination of the RNA samples, a WSSV genomic DNA-specific primer pair, IC-F2/IC-R3 (Liu et al. 2005), derived from an intergenic region of the WSSV genome, was also used as a quality control. The primer sequences are listed in Table 2. Positive control templates were prepared from the WSSV-TW strain.

Construction of Plasmid Standards for Real-Time PCR

The DNAs from the WSSV-challenged A. royi and M. lusoria were also analyzed by real-time PCR for quantification of WSSV loads following the methods described by Dhar et al. (2002). A. royi and M. lusoria 18S rRNA and WSSV ICP11 partial gene fragments were amplified by PCR using the primer pairs 18SF1/18SR1 and ICP11-F/ICP11-R (listed in Tables 1 and 2). The gene fragments were then cloned into the pGEM-T-Easy vector (Promega) and used to construct the plasmids pGEM/Apocy, pGEM/Meret, and pGEM/ICP11, respectively. The plasmid DNAs were dissolved in double-distilled water. Concentrations were determined by spectrophotometry and plasmid copy numbers were calculated. The plasmid solutions were then serially diluted and served as the DNA quantity standards in real-time PCR for quantification of the WSSV copy numbers in the challenged specimens.

Table 1 Primers used for nested WSSV diagnostic PCR in this study

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequences</th>
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<tbody>
<tr>
<td>146F1</td>
<td>5′-ACTACTAACTTCAGCCTATCTAG-3’</td>
</tr>
<tr>
<td>146R1</td>
<td>5′-TAATGCCGGGTTGTAATGTCTTACGA-3’</td>
</tr>
<tr>
<td>146F2</td>
<td>5′-GTAACCTGCCCTTCATCCTCA-3’</td>
</tr>
<tr>
<td>146R2</td>
<td>5′-TACGGCGAGCTGCTGCACTCTTGT-3’</td>
</tr>
<tr>
<td>18SF1</td>
<td>5′-GCTTCCACTTACCTCGTTCTTCT-3’</td>
</tr>
</tbody>
</table>

Real-Time PCR Quantification of WSSV Loads in Challenged A. royi and M. lusoria

Thermal cycling for the quantitative real-time PCR was performed on the ABI PRISM 7300 Real-Time PCR system (Applied Biosystems) and used power SYBR Green PCR Master Mix (Applied Biosystems) as recommended by the supplier. Real-time PCR was applied to the plasmids and WSSV-challenged A. royi and M. lusoria genomic DNAs described above. The primers used for real-time PCR analysis for WSSV were designed based on the sequences of the cloned segment of the WSSV ICP11 gene using Primer Express v.2.0 software (Applied Biosystems), and a universal primer pair for the 18S rRNA gene provided with the PCR Master Mix was used for A. royi and M. lusoria 18S rRNA genes. These primers are listed in Table 2. Known concentrations of plasmid DNAs specific to the WSSV ICP11 gene or to the A. royi and M. lusoria 18S rRNA genes were used to construct standard curves. Quantification of the WSSV amplicons or the 18S rRNA genes was accomplished by measuring the cycle threshold (Ct) value. Background fluorescence was monitored using ROX dye as part of the reaction mixture. All of the above methodology was based on Dhar et al. (2002).

Transmission of WSSV from Challenged M. lusoria to L. vannamei

Gills taken from M. lusoria at 72 h after challenge with the 500× diluted WSSV inoculum were fed to L. vannamei. Two other groups of shrimp fed with heavily WSSV-infected L. vannamei tissues and WSSV-free M. lusoria gills, respectively, served as controls. At 72 hpc, ten shrimp from each group were sampled and subjected to nested WSSV diagnostic PCR.

Results

Detection of the WSSV Genome in Challenged A. royi

Nested WSSV diagnostic PCR was used to detect the presence of WSSV DNA in the challenged A. royi at 0, 3,
24, 48, and 72 hpc. As shown in Fig. 3a, the samples collected at 72 hpc were one-step PCR-positive. In the nested PCR, the viral DNA was detected in both the 48- and 72-hpc samples (Fig. 3b). Amplification of the 18S rRNA gene (Fig. 3c) confirmed that the extracted DNA was of good quality.

Temporal Transcription Analysis of WSSV Gene Expression in Challenged A. royi

The expression profiles of WSSV \(ie1\), \(dnapol\), \(vp664\), and \(vp28\) in the \(A. \) royi at various times after WSSV challenge were analyzed by RT-PCR (Fig. 4). The immediate early gene \(ie1\) and early gene \(dnapol\) were both transcribed as early as 3 hpc and continued to be found through to 72 hpc. The transcripts of the late genes \(vp664\) and \(vp28\) were first detected at 24 hpc, after which they continued to be found to 72 hpc. The quantity of the total RNA templates was confirmed by the 18S rRNA gene control (Fig. 4e), and the absence of WSSV DNA contamination was confirmed by the WSSV genomic DNA-specific primer pair derived from an intergenic region of the WSSV genome (Fig. 4f).

Detection of the WSSV Genome in Challenged M. lusoria

The nested WSSV diagnostic PCR was also performed on the WSSV-challenged \(M. \) lusoria to monitor the presence of WSSV DNA. DNAs from \(M. \) lusoria treated with different concentrations of viral inocula were collected at 0, 3, 24, 48, and 72 hpc. As shown in Fig. 5a, first detection of the viral DNA by first-step PCR amplification with the primer pair 146Fl/146Rl was at 24 hpc for the 500× dilution treatment and at 48 hpc for the 1,000× and 2,000× dilution treatments. The good quality of the extracted DNA was confirmed by successful amplification of the 18S rRNA gene fragments (Fig. 5b). No WSSV-positive signal was observed in any of the samples collected at 0 and 3 hpc.

Temporal Transcription Analysis of WSSV Gene Expression in WSSV-Challenged \(M. \) lusoria

At various time points after \(M. \) lusoria were challenged by immersion in the 500× diluted viral inoculum, samples were taken and the expression profiles of WSSV \(ie1\), \(dnapol\), \(vp664\), and \(vp28\) were analyzed by RT-PCR. None of the analyzed WSSV genes was expressed in the WSSV-challenged \(M. \) lusoria (data not shown).

Real-Time PCR Quantification of WSSV Loads in Challenged A. royi and M. lusoria

In \(A. \) royi challenged by the 500× diluted WSSV inoculum, substantial quantities of the virus were detected at 72 hpc (Fig. 6). In \(M. \) lusoria challenged by the 500× diluted viral inoculum, WSSV genomic DNA was first detected at

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5′–3′)</th>
<th>Usage</th>
</tr>
</thead>
</table>
| \(ie1\) | \(ie1\)-F: 5′-GACTCTACAAATCTCTTTGCCA-3′  \\
|       | \(ie1\)-R: 5′-CTACCCCTGCACCAATTGCTAG-3′ | RT-PCR      |
| \(dnapol\) | \(dnapol\)-F: 5′-TGGGAAGAAAGATGCGAGAG-3′  \\
|       | \(dnapol\)-R: 5′-CAGGCCGAGTAGGATGAC-3′ | RT-PCR      |
| \(vp664\) | 419-F: 5′-CTCCTTACATCTGAGGAAAT-3′  \\
|       | 419-R: 5′-TTGGGACGTACTGGAAGA-3′ | RT-PCR      |
| \(vp28\) | vp28-F: 5′-CTGCTTGTATTGCTGTATTT-3′  \\
|       | vp28-R: 5′-CAGTGGCCGAGTAGGATGAC-3′ | RT-PCR      |
| intergenic | IC-F2: 5′-CATGACTTAAATTGTAAGTCG-3′  \\
|       | IC-R3: 5′-GAATGATTGGTGGCTGTAAGGAC-3′ | RT-PCR      |
| ICP11 | ICP11-F: 5′-TGGGACCTTCCCAGACTGACG-3′  \\
|       | ICP11-R: 5′-TTATTCTTGTTTGCGACAAT-3′ | Cloning     |
| ICP11 | ICP11-F-real: 5′-AGGCACTGACAAGTGTCTAGA-3′  \\
|       | ICP11-R-real: 5′-AATTCTTCGATGCCTTCATTGA-3′ | Real-time PCR |

![Fig. 3](image-url) Amplification of WSSV DNA in experimentally challenged \(A. \) royi using WSSV nested diagnostic PCR. a First-step PCR amplification products (1,447 bp) using the 146F1/146R1 primer pair. b Nested PCR amplification products (941 bp) using 146F2/R2. c \(A. \) royi 18S rRNA gene fragments amplified using the primer pair 18SF1/18SR1 (617 bp) to check the quality of the templates. Lane headings show times post-challenge (h). Two samples were taken for each time point. The arrows indicate the size of the amplicon for each PCR.
24 hpc. At 48 and 72 hpc, the viral load increased substantially (Fig. 7).

Transmission of WSSV from Challenged *M. lusoria* to *L. vannamei*

To monitor the transmission of WSSV, samples were taken from shrimp fed with WSSV-challenged *M. lusoria* and subjected to nested WSSV diagnostic PCR. As shown in Fig. 8, all the shrimp fed with WSSV-challenged *M. lusoria* gills or WSSV-infected shrimp tissues (positive controls) were detected as first-step PCR-positive. Shrimp fed with WSSV-free *M. lusoria* gills were used as negative controls and were all WSSV-negative by nested WSSV diagnostic PCR (data not shown).

**Discussion**

An understanding of how pathogenic agents are transmitted is crucial in both aquaculture and natural resource management. It has been reported that by ELISA and PCR detection, positive signals of WSSV proteins or genomic DNA were found in samples of copepods from the natural environment and from WSD epizootic shrimp-rearing ponds (Huang et al. 1995; Lo et al. 1996a; He et al. 1999; Liu et al. 2000). Recently, a bioassay performed by feeding...
Marsupenaeus japonicus (Perez Farfante and Kensley 1997) with experimentally WSSV-challenged Nitocra sp. copepods further demonstrated the successful transmission of WSSV and subsequent infection of the experimental shrimp (Zhang et al. 2008). Although all of these reports demonstrated the ability of copepods to carry WSSV viral particles, until now, it remained unclear whether the copepods were acting as a host of WSSV or simply as a mechanical vector for the transmission of this virus. Here, time course studies of WSSV-challenged A. royi showed that substantial quantities of WSSV genomic DNA were detected at 72 hpc (Fig. 6) and that a virus load corresponding to a heavily infected state (first-step PCR amplification-positive) was also observed at this time point (Fig. 3). Furthermore, in the challenged A. royi, the time course expression profiles of four WSSV genes closely matched the expression profiles of these genes in WSSV-infected shrimp (Fig. 4; for comparison, please refer to Liu et al. 2005). These results indicate that the copepod A. royi is a WSSV host and that it can become heavily infected.

When a pathogenic agent is shed into water, its pathogenicity can be reduced by a number of factors, including dilution, inactivation by UV light, or inactivation by other physical or chemical means (Noble and Fuhrman 1997; Sinton et al. 2002; Wilhelm et al. 2003). Other factors, such as attachment to suspended particles and uptake by filter-feeding organisms can act to increase pathogenicity (Noble and Fuhrman 1997; Lees 2000). Bivalve mollusks are filter-feeding animals that can filter large amounts of water daily (Galtstoff 1964). If pathogenic microorganisms are present in the water, bivalve mollusks may accumulate the pathogens to levels considerably greater than in the surrounding water (Metcalfe et al. 1979; Rippey 1994). The bioaccumulation and elimination kinetics of the pathogenic agents by bivalve mollusks vary with the species of shellfish (Cabelli and Heffernan 1970) and types of microorganism (Burkhardt et al. 1992). Bivalve mollusks have already been implicated in the spread of various pathogens, and a number of human virus transmission routes have been associated with shellfish (Lees 2000). Presently, however, our knowledge of the interactions between shellfish and shrimp viruses is much more limited. Here, when the hard clam M. lusoria was experimentally challenged with WSSV, we found that large amounts of virus were detected at 24 hpc in samples treated with the 500× diluted viral inoculum (Fig. 5). At 48 hpc, samples tested first-step PCR positive even after treatment with more diluted viral inoculum. The WSSV virus load was also markedly higher after 48 h of immersion in 500× diluted viral inoculum (Fig. 7). However, no WSSV gene expression was observed in these challenged bivalve mollusks. We therefore conclude that M. lusoria could effectively accumulate WSSV but not be infected by this virus.

PCR-based methods are increasingly used for the detection of aquatic pathogens. These methods have become standardized and are often available as commercial kits that are recommended by the OIE Aquatic Manual. However, although PCR methods are useful in providing reliable diagnoses, they are based simply on the presence or absence of the pathogen’s genomic DNA, and they are unable to determine the status of the pathogen in the host. This status can, however, be determined by monitoring the pathogen’s gene expression profiles using RT-PCR. This technique is able to distinguish between contamination/accumulation as opposed to actual infection, and it is applicable not only to WSSV but also to other viruses. Here, we have successfully demonstrated the usefulness of RT-PCR in clarifying the relationship between WSSV and two vector species, A. royi and M. lusoria. In the present study, we have shown that both A. royi and M. lusoria are
able to carry large amounts of WSSV and that shellfish, like copepods, are able to transmit the virus to shrimp, leading to infection and outbreaks of WSD. We have further shown that the virus is able to infect A. royi and successfully replicate in this copepod host. Clearly, in the future, these two animals should both be included as part of the aquatic animal health surveillance program for WSD. Moreover, because of its ability to accumulate large amounts of WSSV in a short time, M. lusoria might be a useful sentinel species for monitoring WSSV farms that culture shrimp and M. lusoria in tandem. This could prove especially useful when the virus is present only in low concentrations.

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References

Escobedo-Bonilla CM, Alday-Sanz V, Wille M, Sorgeloos P, Pensaert M. lusoria—116
Mortensen SH, Hjeltnes B, Rosedoth O, Krogsrud J, Christie KE (1990) Infectious pancreatic necrosis virus, serotype N isolated from Norwegian turbot (Scopthalmus maximus), halibut (Hippoglossus hippoglossus) and scallops (Pecten maximus). Bull Eur Assoc Fish Pathol 10:42–43


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