


# Quantitative analysis of the dose–response of white spot syndrome virus in shrimp

Thuy T. N. Ngo<sup>1,2,3</sup> | Alistair M. Senior<sup>4</sup> | Antica Culina<sup>5</sup> | Eduardo S. A. Santos<sup>6</sup> | Just M. Vlak<sup>2</sup> | Mark P. Zwart<sup>1,7,8</sup> 

<sup>1</sup>Quantitative Veterinary Epidemiology Group, Wageningen University and Research, Wageningen, The Netherlands

<sup>2</sup>Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

<sup>3</sup>Research Institute for Aquaculture No. 2, The Ministry of Agriculture and Rural Development, Ho Chi Minh City, Vietnam

<sup>4</sup>Charles Perkins Centre, and School of Mathematics and Statistics, University of Sydney, Sydney, New South Wales, Australia

<sup>5</sup>Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

<sup>6</sup>BECO do Departamento de Zoologia, Universidade de São Paulo, São Paulo, Brazil

<sup>7</sup>Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-UPV, València, Spain

<sup>8</sup>Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

## Correspondence

Mark P. Zwart, Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands.

Email: m.zwart@nioo.knaw.nl

## Abstract

White spot syndrome virus (WSSV) is an important cause of mortality and economic losses in shrimp farming. Although WSSV-induced mortality is virus dose dependent and WSSV infection does not necessarily lead to mortality, the relationships between virus-particle dose, infection and mortality have not been analysed quantitatively. Here, we explored WSSV dose–response by a combination of experiments, modelling and meta-analysis. We performed dose–response experiments in *Penaeus vannamei* postlarvae, recorded host mortality and detected WSSV infection. When we fitted infection models to these data, two models—differing in whether they incorporated heterogeneous host susceptibility to the virus or not—were supported for two independent experiments. To determine the generality of these results, we reanalysed published data sets and then performed a meta-analysis. We found that WSSV dose–response kinetics is indeed variable over experiments. We could not clearly identify which specific infection model has the most support by meta-analysis, but we argue that these results also are most concordant with a model incorporating varying levels of heterogeneous host susceptibility to WSSV. We have identified suitable models for analysing WSSV dose–response, which can elucidate the most basic virus–host interactions and help to avoid underestimating WSSV infection at low virus doses.

## KEYWORDS

dose–response, infection, meta-analysis, modelling, shrimp, white spot syndrome virus

## 1 | INTRODUCTION

In the 1990s, shrimp aquaculture was devastated by a new pandemic disease, with a causal agent named white spot syndrome virus (WSSV; Escobedo-Bonilla et al., 2008; Walker & Mohan, 2009). WSSV is a relatively novel virus with a large dsDNA genome (van Hulst, Witteveldt, Peters, et al., 2001; Yang et al., 2001), a wide

host range (Escobedo-Bonilla et al., 2008) and high virulence in cultured shrimp (Walker & Mohan, 2009). Here, we understand virulence to be a pathogen-induced reduction in host fitness. Therefore, a simple and highly relevant indicator of virulence in this pathosystem is virus-induced host death prior to shrimp maturity. Viral virulence depends on many factors, a number of which have been explored for WSSV. There is great variation in the virulence of

different WSSV genotypes (Laramore, Scarpa, Laramore, & Lin, 2009; Marks, van Duijse, Zuidema, van Hulten, & Vlak, 2005; Pradeep, Karunasagar, & Karunasagar, 2009; Waikhom, John, George, & Jeyaseelan, 2006; Zwart, Dieu, Hemerik, & Vlak, 2010). Moreover, during its spread throughout farms in Asia, WSSV appears to have evolved a smaller genome and higher virulence (Dieu et al., 2004; Marks et al., 2005; Zwart et al., 2010). Furthermore, differences in virulence between WSSV genotypes appear to matter in shrimp farming (Hoa, Zwart, Phuong, de Jong, & Vlak, 2012; Hoa, Zwart, Phuong, Vlak, & de Jong, 2011), although in other studies, the same marker loci used do not appear to be linked to disease outbreaks (Walker et al., 2011). Other factors that are known to affect WSSV virulence are host species (Escobedo-Bonilla et al., 2008; Waikhom et al., 2006), water temperature (Rahman et al., 2006, 2007), interactions with bacteria (Phuoc et al., 2008), different levels of replication in different host tissues (Rahman et al., 2008) and previous exposure to WSSV. The latter can result in long-lived protection to the virus (Johnson, van Hulten, & Barnes, 2008; Venegas, Nonaka, Mushiake, Nishizawa, & Muroga, 2000). In summary, the virulence of WSSV does not appear to be constant over time and space (Flegel, Nielsen, Thamavit, Kongtim, & Pasharawipas, 2004). As with many other diseases, there is a pressing need to identify and understand those factors modulating WSSV virulence, to determine how these factors interact, and to have models to explain and predict the advance of the disease in a population in space and time.

One important factor in determining whether WSSV infection and virulence will occur is the dose of virus particles to which a host has been exposed. For many viruses, hosts must be exposed to a large number of virus particles to become infected, as the probability of infection per virus particle is very small (Zwart, Daròs, & Elena, 2011; Zwart & Elena, 2015). Whereas very high virus doses may infect all hosts, in practice exposure to a broad range of doses will lead to infection in only some hosts. A better understanding of the relationship between dose and response can be useful for identifying when host organisms will be at risk of disease and for understanding the effects of different interventions to mitigate the effects of disease outbreaks. When mechanistic models of the dose–response are fitted to experimental data, this approach also can be useful for better understanding of the infection process. For example, if virus particles cooperate with each other during the infection process, then increases in dose will have disproportionate effects on the rate of host infection, leading to a steep dose–response. Observation of a steep dose–response has been crucial to demonstrating the strong cooperation between virus particles that stem from packaging of different genome segments into separate virus particles in some plant (Fulton, 1962; Sánchez-Navarro, Zwart, & Elena, 2013) and animal viruses (Ladner et al., 2016).

Many studies have shown that the rate of WSSV infection depends on virus particle dose (Escobedo-Bonilla et al., 2005; Laramore et al., 2009; Marks et al., 2005; Prior, Browdy, Shepard, Laramore, & Parnell, 2003). It has also been suggested that WSSV virus particles might be acting independently during the infection process because dose–response is similar to predictions of the independent

action hypothesis (IAH) model (Dieu, Zwart, & Vlak, 2010). This model states that each virus particle can be assigned a nonzero probability of infection, and that virus particles act independently, leading to clear predictions for the shape of the dose–response (Druett, 1952; Regoes, Hottinger, Sygnarski, & Ebert, 2003; Zwart et al., 2009). However, none of the WSSV studies rigorously compared experimental data to mechanistically interpretable models, nor was model selection performed to identify which model is best supported by the empirical data. Furthermore, not all shrimp that become infected with WSSV will die (Flegel, 2007; Johnson et al., 2008; Venegas et al., 2000). One study has shown systematically that sublethal infection is infrequent in laboratory challenge experiments (Escobedo-Bonilla et al., 2005), although it is quite common in the field (Hoa, Zwart, Phuong, Oanh, et al., 2011). The relationship between dose, infection and mortality has also not been explored systematically or quantitatively.

Here, we set out to provide a quantitative description of the WSSV dose–response relationship. By performing model selection on a set of mechanistically interpretable models, we wanted to identify the underlying mechanisms that may give rise to this relationship. Our contribution to these issues is threefold. First, we performed dose–response experiments and determined the infection status of all shrimp in the experiment, as well as recording mortality. We could therefore simultaneously consider dose–response on two levels: systemic infection and host mortality. Furthermore, the set-up described here is different from previous reports because the communal housing of shrimp allowed waterborne transmission, and we needed to include these effects in our models. Second, we fitted a number of generally applicable and mechanistically interpretable models of dose–response to these data using a maximum likelihood approach, and then performed model selection. Third, to gauge the generality of our conclusions, we performed a meta-regression analysis on reanalysed published dose–response data for WSSV in shrimp.

## 2 | MATERIALS AND METHODS

### 2.1 | Shrimp and virus isolates

Specific pathogen-free *Penaeus vannamei* (Boone, 1931) postlarvae (PL) were purchased from Gold Coin Singapore, imported to the Netherlands and reared by the Aquatic Research Facility at Wageningen University. *Orconectes limosus* (Rafinesque, 1817) were obtained from local fishers from the Meuse River and used for virus amplification. Shrimp were screened for common viral diseases by polymerase chain reaction (PCR) or reverse transcription (RT) PCR (Witteveldt, Cifuentes, Vlak, & van Hulten, 2004). Shrimp were kept in tanks with heating (28°C), continuous aeration and individual filter systems (Eheim).

For all experiments, we used purified WSSV virus particles (Xie, Li, Xu, & Yang, 2005) from isolate VN-T (Dieu et al., 2004). For dose–response experiment #1, the virus was amplified in *O. limosus* prior to purification, and for experiment #2, *P. vannamei* was used for amplification.

## 2.2 | Dose–response experiments

For the dose–response experiments, individual shrimp were kept in cages within a 180 L aquarium. On each side, the 8 × 8 cm slots had a round opening with a 6 cm diameter covered with a fine metal mesh (1 mm), allowing water to flow freely between the slots. Shrimp in these cages and aquaria were randomly assigned to the different treatments (i.e., different virus doses or mock-infected controls). Shrimp PL10–12 were weighed and then intramuscularly injected with 10 μl of WSSV virus particles or phosphate-buffered saline (PBS) for controls. Injections were performed with a 1.5 ml BD Pen (Becton Dickinson) and 28G 1/2" NovoFine needles (Novo Nordisk). A later developmental stage (PL of 1–3 g in both experiments) was chosen because these shrimp can be injected intramuscularly, and because at this stage disease and mortality occur quickly, making experiments more tractable. The appearance, behaviour and mortality of shrimp were observed daily, and dead shrimp were removed immediately and stored at –20°C. Hence, our set-up with the cages prevented cannibalism of dead shrimp, but not waterborne transmission of the virus. After 10 days, all surviving shrimp were collected and stored. For experiment #1, cohorts of 13 shrimp each were inoculated with a 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> or 10<sup>6</sup> fold dilution of purified WSSV virus particles amplified in *O. limosus*, or PBS only for mock-inoculated controls. For experiment #2, cohorts of 17 shrimp each were inoculated with a 10, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> or 10<sup>6</sup> dilution of WSSV virus particles amplified in *P. vannamei*, or PBS only. Shrimp taken from the same cohort were used for experiments #1 and #2, although experiment #1 was performed first and the shrimp were smaller.

## 2.3 | PCR detection of WSSV

The infection status of all shrimp from the dose–response experiments was determined by PCR. To avoid environmental contamination with WSSV DNA, a sample of muscle tissue was used for DNA extraction. A crude extract of DNA was used as template for two separate Taq-based PCR reactions with host- and virus-specific primers (Witteveldt et al., 2004; host-specific primers for 16S ribosomal RNA: 5'-GTGCGAAGGTAGCATAATC and 5'-CTGCTGCAACATAA GGATAC, WSSV-specific primers for VP26: 5'-ATGGAATTTGGC AACCTAACAAACCTG and 5'-GGGCTGTGACGGTAGAGA). Host-specific primers were included as a positive control for DNA extraction and PCR. PCR products were resolved on a 0.7% agarose gel prestained with ethidium bromide.

## 2.4 | Dose–response models

To model dose–response, we took the simplest mechanistic model of virus infection as a starting point: the IAH model (Druett, 1952; Regoes et al., 2003; Zwart et al., 2009). The dose–response prediction of this model is obtained from the zero term of the Poisson distribution for the number of infecting virus particles, which represents those hosts that have not been infected by the virus

(Zwart et al., 2009). The rate of infection  $I$  is then  $I = 1 - S = 1 - e^{-\lambda} = 1 - e^{-\rho n}$ , where  $S$  is survival,  $\lambda$  is the number of infecting virus particles,  $\rho$  is the probability of infection for each virus particle and  $n$  is the number of virus particles in the inoculum. In this case, we do not know the actual virus particle dose, but we know the relative viral dose as the different doses were obtained from serial dilutions of a WSSV stock. We therefore set the dose of the virus stock to an arbitrary high value (10<sup>9</sup> virus particles). The same convention also was used for all analysis and presentation of data throughout this study.

To apply this model to our experimental data, we must make three considerations. First, in our experiments, shrimp can become infected due to the intramuscularly injected virus particles, or due to waterborne transmission of the virus from other shrimp that have become infected during the experiment, as the shrimp are housed communally. The waterborne WSSV dose to which a shrimp is exposed probably will increase over time, and the probability of infection for each virus particle in the water will probably be lower than for injected virus particles (Soto & Lotz, 2001). As the waterborne virus particle load will be approximately the same for all shrimp sharing the same water, and both dose and infection probability are unknown, we simply introduce a second infection term analogous to  $\lambda$  to represent waterborne transmission ( $\omega$ ):

$$I = 1 - e^{-\rho n - \omega} \quad (1)$$

Next, we also want to model the rate of mortality, because we measured both infection and mortality for the same group of hosts. If we assume that infection does not necessarily lead to host death, and that the IAH principle also applies to this level of infection, we can introduce a probability that an infecting virus particle causes host death ( $\varphi$ ). We consider this probability the same for infection resulting from intramuscular infection or waterborne transmission, in which case host mortality ( $M$ ) is:

$$M = 1 - e^{-\varphi(\rho n + \omega)} \quad (2)$$

In reality, for infection resulting from virus particles transmitted by waterborne transmission, the probability of host death might be smaller: These secondary infections will have started later than infections caused by the injected virus particles, and therefore, there will be less time for the virus to kill the host. However, as a first approximation and to avoid overparameterising the model, we ignore this effect.

Finally, as in many cases, the data did not support the IAH model, more complex models of infection must be considered (Ben-Ami, Regoes, & Ebert, 2008; Regoes et al., 2003; van der Werf, Hemerik, Vlak, & Zwart, 2011). We consider two alternative models of infection: (a) a model incorporating dose-dependent interactions between virus particles, the dose-dependent action (DA) model; and (b) a model allowing for differences between hosts in their susceptibility to the virus, the heterogeneous host susceptibility (HHS) model.

For the DA model, given similarities in the dose–response observed for infection and mortality for our data set—and to avoid

an overparametrized model—we chose to add dose dependence at the infection level. These dose-dependent effects on infection will carryover on the rate of mortality, and as a result, the shape of both responses will be affected. Also, as the infection routes for injected and waterborne virus particles are different, and the waterborne virus particle dose is assumed to be constant, we chose to only have dose-dependent effects stemming from the dose of injected virus particles. Therefore, following (Regoes et al., 2003), the rate of infection under the DA model is:

$$I = 1 - e^{-\rho n^\kappa - \omega} \quad (3)$$

where  $\kappa$  is a constant that determines what type of dose-dependent effects will occur. When  $\kappa < 1$ , there are antagonistic interactions between virus particles; as the dose is increased, it becomes harder for each virus particle to infect, and hence, the dose–response becomes more gradual than for IAH. When  $\kappa > 1$ , there are synergistic interactions; as the dose is increased, it becomes easier for each virus particle to infect, and hence, the dose–response is steeper than for IAH. When  $\kappa = 1$ , the model collapses to the IAH model. As the number of infecting virus particles is dose dependent for the DA model, the response of  $M$  will also be dose dependent if all else is kept equal.

For the HHS model, “frailty” models have been used to predict the relationship between dose and infection, assuming the distribution of host susceptibility follows a  $\Gamma$ -distribution (Ben-Ami et al., 2008). Our approach is conceptually similar to the DA model: For parsimony, we introduce heterogeneity only in the infection step, so that the effects also carryover from infection to mortality. Given that  $S = \left(\frac{1}{1 + \rho\nu n}\right)^{1/\nu}$  for this infection model (Ben-Ami et al., 2008) and the occurrence of waterborne transmission in our set-up, the dose–response is as follows:

$$I = 1 - e^{-\omega} \left(\frac{1}{1 + \rho\nu n}\right)^{1/\nu} \quad (4)$$

where  $\nu$  is a constant that determines the variance of the distribution of host susceptibilities ( $\rho\nu^2$ ). Differences in host susceptibility can only result in a more gradual dose–response: For increasing values of  $\nu$ , the dose–response becomes more gradual, whereas when  $\nu$  approaches zero, the shape of the dose–response becomes identical to the IAH prediction. By including the probability that an infecting virus particle causes host death in the terms for waterborne transmission and the injected inoculum, the relationship between dose and mortality is  $M = 1 - e^{-\varphi\omega} \left(\frac{1}{1 + \rho\varphi\nu n}\right)^{1/\nu}$ .

## 2.5 | Model fitting and model selection

For the IAH model, parameters  $\rho$ ,  $\varphi$  and  $\omega$  must be estimated. For the DA model, parameters  $\rho$ ,  $\varphi$ ,  $\omega$  and  $\kappa$  must be estimated. For the HHS model, parameters  $\rho$ ,  $\varphi$ ,  $\omega$  and  $\nu$  must be estimated. For the experimental data, three final states were observed as follows: (a) survival ( $S$ ): Shrimp that survive and are not infected by WSSV ( $S$ ); (b) exposure ( $E$ ): Shrimp that are infected by WSSV and survive to the end of the experiment; and (c) death ( $M$ ): Shrimp that die and

are infected by WSSV. There were no shrimp that died but were negative for WSSV detection, a host state that is also not possible for our infection models (per definition the probability  $\varphi \leq 1$ ). The IAH and DA models can be used to predict the number of animals in each state as a function dose, given  $S = 1 - I$  and  $E = I - M$ . The likelihood of observing a given number of shrimp in the  $M$ ,  $E$  or  $S$  states at the end of the experiment ( $X_1, X_2, X_3$ ) follows a multinomial distribution with probabilities  $p_1$ ,  $p_2$  and  $p_3$  ( $\sum_{i=1}^3 p_i = 1$ ). A particular realization  $(x_1, x_2, x_3)$  has a multinomial probability:

$$P(X_1 = x_1, X_2 = x_2, X_3 = x_3) = \frac{\left(\sum_{i=1}^3 x_i\right)!}{\prod_{i=1}^3 x_i!} \prod_{i=1}^3 p_i^{x_i}. \quad (5)$$

We used a stochastic hill-climbing algorithm to minimize the negative log likelihood (NLL), with  $10^3$  searches from randomly chosen points in a broad range of plausible parameter-value space. We then used the Akaike information criterion (AIC) for model selection, an approach that weighs both the fit and the number of model parameters in choosing which model is best supported by the data. Note that the DA and HHS models add one model parameter, and that the number of model parameters is similar for all models. From the difference in AIC scores between the models ( $\Delta\text{AIC}$ ), we determined the Akaike weight (AW), an indicator of the relative likelihood of a model given the set of models compared (Johnson & Omland, 2004). We also performed model selection on the model fittings for the two experiments, to gauge overall support for the different models (Navakatikyan, 2007). All analyses were performed in R 3.3 (R Core Team, 2016; RRID:SCR\_001905), with custom scripts.

## 2.6 | Survival analysis

The status of shrimp in the dose–response experiments was scored daily, making it possible to analyse whether there are any effects of dose on the time until death. We tested for significant differences in time until death between doses using the log rank test, pooled over strata and using only the data from those animals that die by the end of the experiment. As infection status was only determined at the end of the experiment due to the invasive sampling method, we can only perform survival analysis for mortality.

## 2.7 | Meta-analysis

We searched the scientific literature for dose–response data on shrimp and WSSV. Studies had to meet the following criteria to be included: (a) use WSSV as an inoculum, using any method of exposure; (b) use shrimp as the challenged host; (c) consider at least three different doses, with a dose range such that infection or mortality is not greater than or less than 0.5 for all doses (i.e., to exclude any data sets with only very high or very low levels of response, which would not allow discrimination between the infection models); (d) consider  $>5$  shrimp per dose; (e) report the numbers of shrimp for the response; and (f) the response should be mortality, or the rate of infection measured in an unbiased manner (i.e., testing of all shrimp, and not a subsample of dead or alive shrimp). If both usable

mortality and infection data were reported (two of 16 data sets), we used only the infection data in this analysis to avoid including the same data set twice. In one data set with both infection and mortality data, there are discrepancies between them: that is, not all dead shrimp are WSSV infected (Laramore et al., 2009). In this case, infection is clearly the better indicator of the presence of WSSV.

We then fitted the IAH, DA and HHS models to the data, but considering only a one-step model (i.e., in effect considering all data sets to be infection data and using the models to estimate  $I$ ). For the reanalysis, we also left out the waterborne transmission term from the models as none of these experiments reported infection or mortality in the mock-inoculated controls. Thus, for the IAH model, only parameter  $\rho$  must be estimated, for the DA model,  $\rho$  and  $\kappa$  must be estimated, and for the HHS model,  $\rho$  and  $\nu$  must be estimated. Given that these data were then reduced to two possible outcomes ( $S$  and  $I$ ), we could use binomial likelihoods to determine the NLL and AIC. A null model with virus dose-independent host mortality ( $I = \theta$ , where  $\theta$  is a constant) was also fit to the data, to allow for estimation of McFadden's pseudo- $R^2$  (subsequently referred to as  $R^2$ ) for each models as  $R^2 = 1 - \ln(L)/\ln(L_0)$ , where  $L$  is the likelihood of the model and  $L_0$  the likelihood of the null model (McFadden, 1973). Given that IAH model is highly constrained, negative  $R^2$  values could be expected in some cases, and these values were reset to zero.

$R^2$  values are bounded at zero and one, but for meta-analysis, effect sizes need to be unbounded. For meta-analysis, we logit transformed  $R^2$  values, that is zero instances were adjusted by 1—maximum value of  $R^2$  (Warton & Hui, 2011). The sampling variance ( $s^2$ ) for each value of logit transformed  $R^2$  was estimated following Equation 6:

$$s^2 = \sqrt{\frac{1}{n}R^2 + \frac{1}{n(1-R^2)}} \quad (6)$$

where  $n$  is the number of shrimp in the experiment (Mengersen & Gurevitch, 2013). To compare  $R^2$  values from different models of infection, we used a multilevel meta-regression (MLMR; Nakagawa, Noble, Senior, & Lagisz, 2017; Nakagawa & Santos, 2012). MLMR was fitted using the "rma.mv" function in the R package *metafor* (Viechtbauer, 2010). Logit transformed  $R^2$  values were fitted as the effect size, and  $s$  as the sampling variance. The study ID was fitted as a random-effect, and the type of infection model as a three-level categorical fixed effect. Estimated differences between the overall mean effect sizes for different infection models with 95% confidence intervals (CIs) excluding zero are considered statistically significant. Logit transformed model estimates are back transformed in places for interpretation.

To explore whether differences among host species may affect the predictive power of the three infection models (i.e., moderator variables), we then fitted random effects meta-regression (REMR) models. Data were split into three subsets, one for each infection model type. For each subset, we fitted two REMRs again with the logit  $R^2$  and its sampling variance ( $s^2$ ) as the effect size. In the first REMR, we fitted the log mean host mass as a linear moderator, and

in the second, we fitted the host species as three-level categorical predictor. Model coefficients (e.g., estimated differences between the overall mean effect sizes for different infection models) with 95% confidence intervals (CIs) excluding zero are considered statistically significant. Logit transformed model estimates are back transformed in places for interpretation.

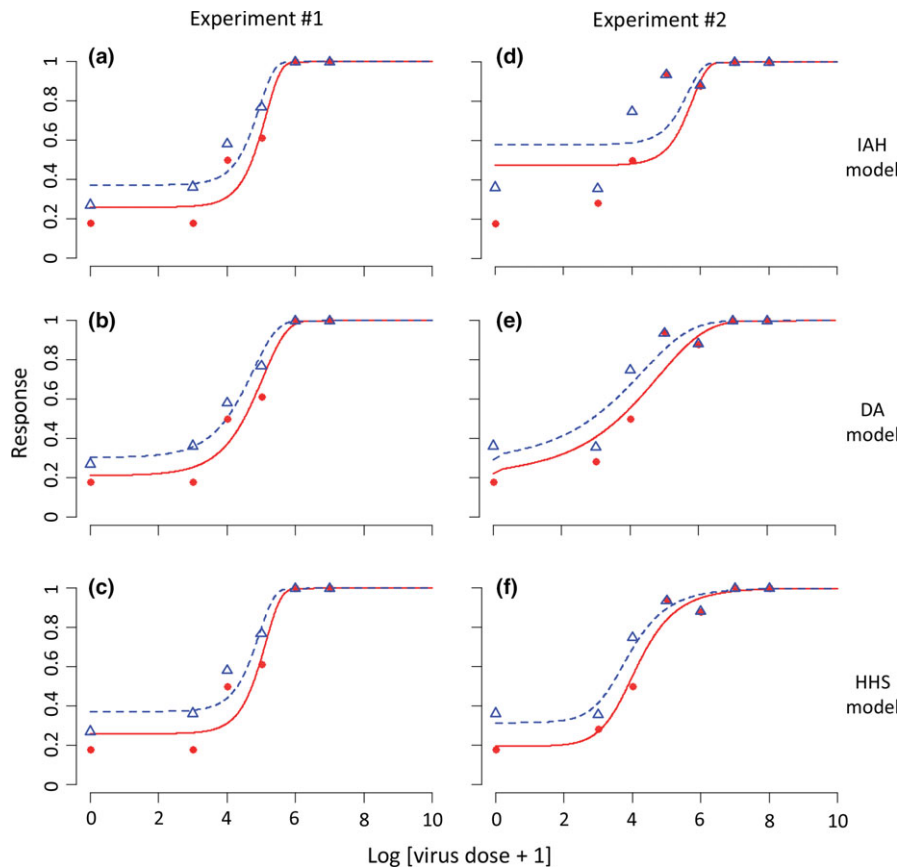
### 3 | RESULTS

#### 3.1 | Experimental data

To investigate the dose–response of WSSV in shrimp, *P. vannamei* PL were infected by intramuscularly injecting 10-fold dilutions of WSSV isolate VN-T (Dieu et al., 2004). Two experiments were performed, the first with isolate VN-T amplified in *O. limosus*, and the second with the same isolate amplified in *P. vannamei*. At the start of the experiment, the weight of shrimp (mean  $\pm$  SD) was  $1.38 \pm 0.46$  g ( $N = 71$ ) for experiment #1, and  $2.37 \pm 0.48$  g ( $N = 107$ ) for experiment #2. Mortality was recorded daily, and the presence of WSSV in all shrimp (whether surviving or dead) was determined by PCR after termination of the experiment (10 days postinfection). At the end of the experiment, the mean length of shrimp was (mean  $\pm$  SD) was  $5.89 \pm 0.85$  cm ( $N = 71$ ) for experiment #1, and  $7.18 \pm 0.54$  cm ( $N = 107$ ) for experiment #2. For both experiments, we observed a similar sigmoidal dose–response for both infection and mortality (Figure 1a, see also Supporting Information Table S1). Some mock-inoculated controls were also found to be infected with WSSV and dead (Figure 1d). This observation confirmed the occurrence of waterborne transmission, which was expected as the shrimp were physically separated by a fine mesh, but water flowed freely between cubicles. We found that some infected shrimp had not died by 10 dpi (Figure 1a,d). The number of shrimp that were both infected and survived until the end of the experiment was low, reaching a maximum of 1/3 of infected shrimp (four of 12 shrimp for second to lowest dilution of the virus amplified in *P. vannamei*). Shrimp from the same cohort kept in separate aquaria experienced no WSSV infection or mortality, indicating that the shrimp population used for the experiment was not infected.

#### 3.2 | Quantitative analysis of dose–response and survival analysis

The IAH and DA dose–response models were fitted to the data with a maximum likelihood method (Figure 1, Table 1). Diverging results were obtained for experiments #1 and #2. For experiment #1—with the virus amplified in *O. limosus*—the IAH model was best supported, although the model fittings and supports were similar for all three models (Figure 1a–c, Table 2). For experiment #2—with the virus amplified in *P. vannamei*—the HHS model was clearly the best fitting and best-supported model, followed by the DA model (Figure 1d–f, Table 2). For this data set, the IAH model had virtually no support, as the model fits very poorly (Figure 1d). Model parameter estimates (i.e.,  $\nu \gg 0$  for the HHS model and  $\kappa < 1$  for the DA model) confirm



**FIGURE 1** Dose–response for white spot syndrome virus (WSSV) in *Penaeus vannamei*. For all panels, the ordinate is  $\log_{10}$ -transformed virus dose + 1 in arbitrary units, with  $10^9$  corresponding to the undiluted virus stock. Note that on this scale, zero represents the mock-infected virus controls. The abscissae are the response, infection or mortality. Open blue triangles indicate the infection data, whereas the dotted blue line indicates the fitted infection model. Filled red circles indicate mortality data, whereas the solid red line indicates the fitted mortality model. Panels a–c represent the same experimental data from experiment #1 (WSSV stock amplified in *Orconectes limosus*), whereas panels d–f represent the same data of experiment #2 (WSSV stock amplified in *P. vannamei*). Panels a and d show the fitted independent action hypothesis (IAH) model, panels b and e show the fitted dependent action (DA) model, and panels c and f show the fitted the heterogenous host susceptibility (HHS) model. Whereas the more complex DA and HHS models do not lead to an appreciable improvement in model fit for the data from experiment #1, there is clearly an improvement for the data of experiment #2. When model selection is performed on the two data sets, the HHS model has the most support (Table 2)

that the response was more gradual than IAH predictions in experiment #2. When the model fits for the two experiments were combined in a single model selection, the HHS model was again better supported than the DA model, whilst there was virtually no support for the IAH model (Table 2). Overall, our experimental data therefore provide most support for the HHS model.

When shrimp mortality over time was plotted, there appears to be an effect of dose on shrimp survival in both experiments. Animals exposed to a higher dose appear to die sooner (Figure 2), and there was a significant effect of dose on time until death in both experiments (Log rank test; experiment #1:  $\chi^2 = 14.266$ ,  $df = 5$ ,  $p = 0.014$ ; experiment #2:  $\chi^2 = 25.570$ ,  $df = 6$ ,  $p < 0.001$ ).

### 3.3 | Meta-analysis of WSSV dose–response

To determine the generality of the results based on our experimental data, a reanalysis and meta-analysis of WSSV dose–response were

**TABLE 1** Estimated model parameters

Data set	Model	$P$	$\phi$	$\Omega$	$K$	$N$
I <sup>a</sup>	IAH	$1.12 \times 10^{-5}$	0.646	0.468	-	-
	DA	$2.00 \times 10^{-4}$	0.661	0.363	0.617	-
	HHS	$1.15 \times 10^{-5}$	0.646	0.468	-	$3.80 \times 10^{-2}$
II <sup>b</sup>	IAH	$2.29 \times 10^{-6}$	0.741	0.871	-	-
	DA	$1.86 \times 10^{-2}$	0.724	0.347	0.309	-
	HHS	$1.86 \times 10^{-4}$	0.575	0.380	-	1.905

Notes. DA: dose-dependent action; HHS: heterogeneous host susceptibility; IAH: independent action hypothesis.

<sup>a</sup>WSSV amplified in *Orconectes limosus*. <sup>b</sup>WSSV amplified in *Penaeus vannamei*.

performed on published data sets. We found 16 data sets, published in six studies (Escobedo-Bonilla et al., 2005; van Hulten, Witteveldt, Snippe, and Vlak 2001; Laramore et al., 2009; Marks et al., 2005;

**TABLE 2** AIC values for the infection and mortality models fitted to our experimental data

Data set	Model	Parameters	NLL	AIC	$\Delta$ AIC	AW
I <sup>a</sup>	IAH	3	11.070	28.139	-	0.536
	DA	4	10.767	29.535	1.395	0.267
	HHS	4	11.066	30.131	1.992	0.198
II <sup>b</sup>	IAH	3	22.980	51.960	15.569	<0.001
	DA	4	17.221	42.442	6.051	0.046
	HHS	4	14.195	36.391	-	0.953
I + II	IAH	6	34.049	80.099	13.577	0.001
	DA	8	27.988	71.976	5.454	0.061
	HHS	8	25.261	66.522	-	0.938

Notes. AIC: Akaike information criterion; AW: Akaike weight; DA: dose-dependent action; HHS: heterogeneous host susceptibility; IAH: independent action hypothesis; NLL: negative log likelihood.

<sup>a</sup>WSSV amplified in *Orconectes limosus*. <sup>b</sup>WSSV amplified in *Penaeus vannamei*.

Motamedi Sedeh et al., 2012; Thuong et al., 2016), that met all our criteria for inclusion in this analysis. The IAH, DA and HHS models were fitted to these data sets, and model selection was performed individually for each data set (Supporting Information Table S2; a summary of the results is given in Table 3). Upon reanalysis of the individual data sets, there were two clear parallels with our own experimental results. First, in some experiments, the IAH model was supported, whereas in others, the more complex DA or HHS models were better supported (Table 3). Second, the dose-response was either similar to IAH, as seen for our experiment #1, or when this model was not supported, more gradual than model predictions, with  $\nu \gg 0$  for the HHS model and  $\kappa < 1$  for the DA model, as seen for our experiment #2 (Table 3).

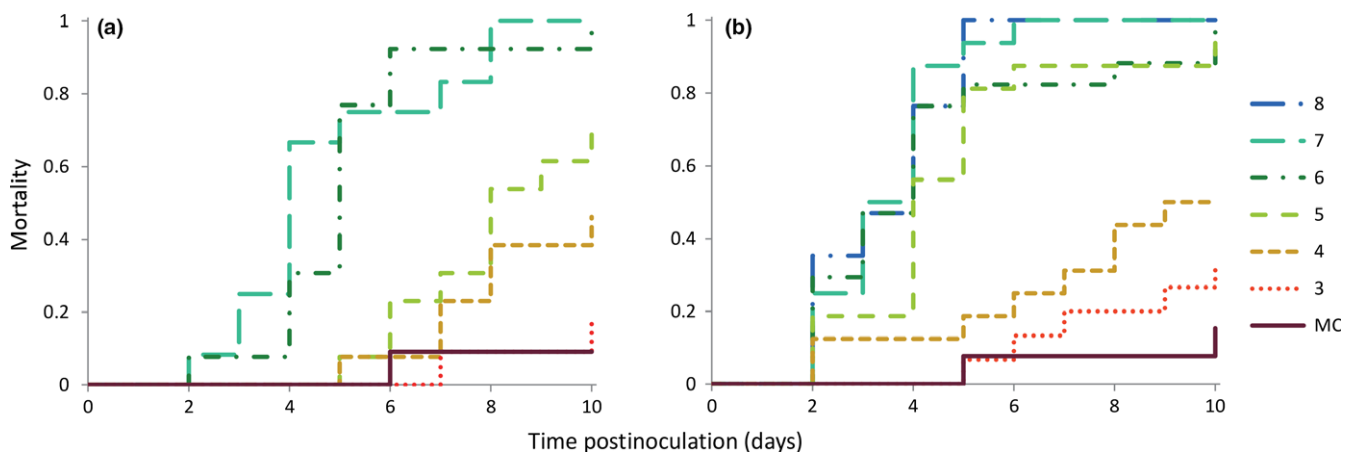
Meta-analysis was used to determine which model accounted for the most variation over all 16 data sets. MLMR indicated that the mean  $R^2$  of the IAH model was 0.547, whereas that for the DA and

HHS models was 0.838 and 0.841, respectively (IAH model logit est. = 0.191, CI = -0.651–1.032; DA model logit est. = 1.644, CI = 0.786–2.501; HHS model logit est. = 1.669, CI = 0.812–2.526). Contrasts among model types indicate that the  $R^2$  of the DA and HHS models are significantly higher on average than that of the IAH model (logit est.<sub>DA-IAH</sub> = 1.453, CI = 0.633–2.273; logit est.<sub>HHS-IAH</sub> = 1.479, CI = 0.659–2.298). However, there was no significant difference between the  $R^2$  of the DA and HHS models (logit est.<sub>HHS-DA</sub> = 0.026, CI = -0.810–0.862). Whilst the meta-analysis highlights that the DA and HSS model are both superior to the IAH model, both models are equally supported. On the one hand, the results of the meta-analysis are similar to our experimental work, as in some experiments, the IAH model is rejected because of the gradual dose-response. On the other hand, the HHS model was not superior to the DA model, as was the case for our own experimental data (Table 2).

We detected positive effects of host mass on  $R^2$  of all three infection models; however, the effect was only statistically significant in the case of the IAH model (IAH model slope est. = 0.835, CI = 0.056–1.614; DA model slope est. = 0.332, CI = -0.056–0.721; HHS model slope est. = 0.297, CI = -0.064–0.658; Figure 3a–c, Supporting Information Table S3). We did not detect any statistically significant differences among the three host species for any of the infection models (Figure 3d,e; Supporting Information Table S3), although very little data are available for *P. semisulcatus*.

## 4 | DISCUSSION

To understand better the WSSV infection process and the risks of infection and mortality in shrimp, we used a combination of dose-response experiments, infection modelling and meta-analysis. We used simple models of infection that can be interpreted mechanistically, allowing for a better understanding of the WSSV infection process.



**FIGURE 2** The relationship between white spot syndrome virus dose and time until death. For both panels, the ordinate is the time postinoculation, and the abscissae are the proportion of mortality. The response for different  $\log_{10}$ -transformed arbitrary doses is denoted by the legend, and MC indicates the mock-inoculated controls. In panel a, the results for experiment #1 are given, and note the highest dose ( $10^8$ ) was not taken. In panel b, the results for experiment #2 are given. For both experiments, there was a statistically significant effect of dose on the time until death

**TABLE 3** Summary of the reanalysis of published data

Study	WSSV isolate <sup>a</sup>	Host <sup>b</sup>	Number <sup>c</sup>	Model parameters <sup>d</sup>		Akaike weight		
				$\kappa$	$\nu$	IAH	DA	HHS
van Hulst, Witteveldt, Peters, et al. (2001)	TH-96 (Thailand)	m	64	0.224	2.692	0	0.007	0.993*
Marks et al. (2005)	TH-96 (Thailand)	m	28	0.603	10 <sup>-7.19</sup>	0.564*	0.220	0.215
	TH-96-II (Thailand)	m	25	0.550	0.933	0.401*	0.380	0.220
Escobedo-Bonilla et al. (2005)	Söderhall (Thailand)	v	60	0.692	0.995	0.143	0.282	0.574*
	Söderhall (Thailand)	v	60	0.759	0.513	0.294	0.379*	0.327
Laramore et al. (2009)	CH1995-1 (China)	v	40	0.339	6.310	0	0.283	0.716*
	CH1995-2 (China)	v	40	0.309	10.233	0.001	0.535*	0.464
	M-M2001 (Mexico)	v	38	0.039	28.840	0	0.511*	0.489
	N2000 (Nicaragua)	v	40	0.427	2.818	0.001	0.570*	0.430
	EOLT2002 (Ecuador)	v	40	0.631	0.912	0.296	0.431*	0.272
	E-L1999 (Ecuador)	v	40	0.851	0.288	0.560*	0.222	0.218
	H2000-1 (Honduras)	v	40	0.851	0.288	0.560*	0.222	0.218
	H2000-2 (Honduras)	v	40	0.490	0.741	0.026	0.520*	0.455
M-LP2001 (Mexico)	v	40	0.296	1.905	0	0.452	0.548*	
Motamedi Sedeh et al. (2012)	- (Iran)	s	84	0.661	0.708	0.313	0.496*	0.191
Thuong et al. (2016)	Söderhall (Thailand)	v	45	1.349	10 <sup>-6.58</sup>	0.520*	0.288	0.192

Notes. DA: dose-dependent action; HHS: heterogeneous host susceptibility; IAH: independent action hypothesis; WSSV: white spot syndrome virus.

<sup>a</sup>Name of the WSSV isolate, with the country of isolation in brackets. <sup>b</sup>Host species used for challenge experiments: m = *Penaeus monodon*, v = *Penaeus vannamei*, s = *Penaeus semisulcatus*. <sup>c</sup>The total number of shrimp used in the experiment. <sup>d</sup>Estimates of model parameters.

\*Model with most support is indicated.

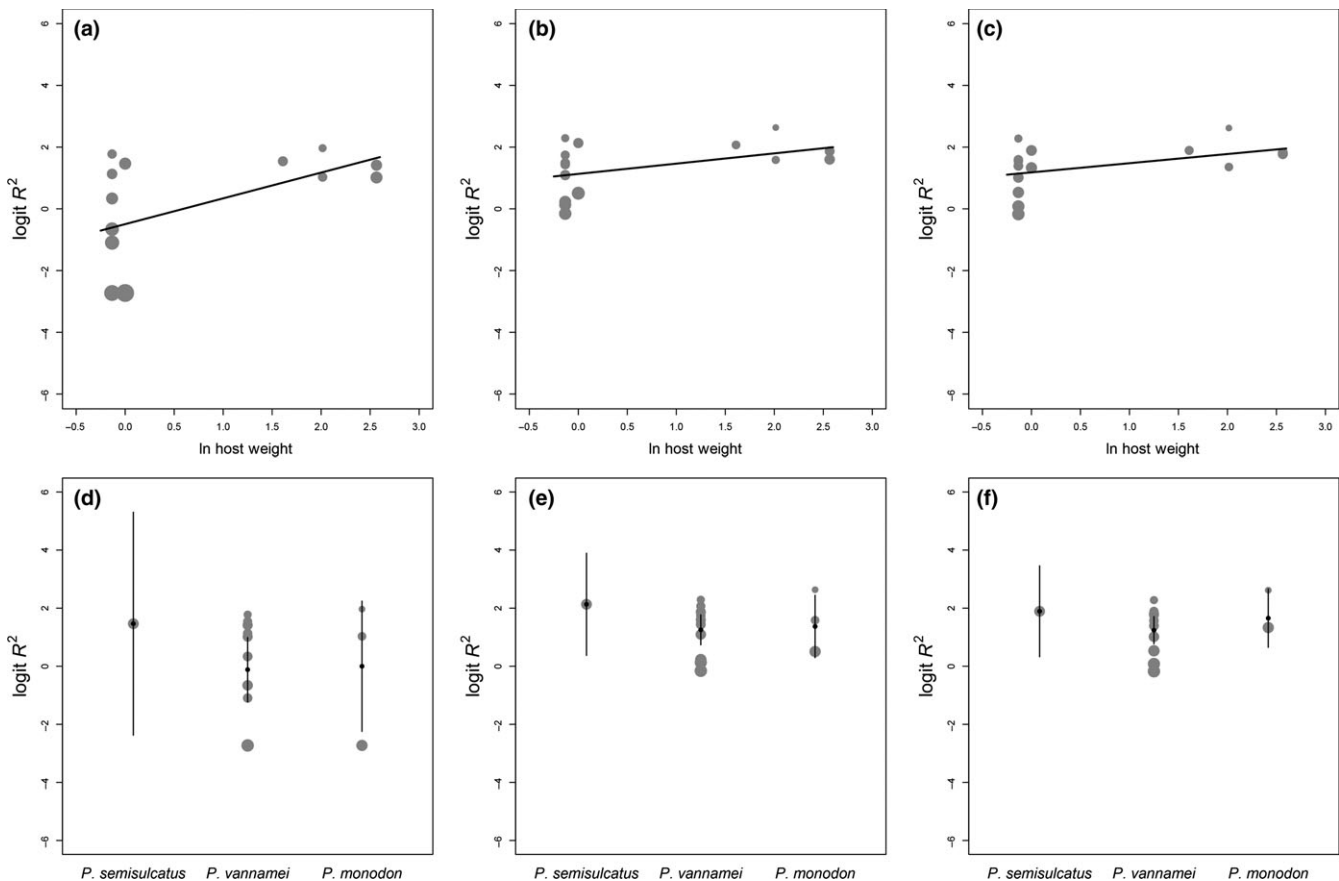
Model selection on the data from our dose–response experiments suggests the most support exists for the HHS model. However, when the data of the two experiments are considered separately, only the data from experiment #2 provided support for this model. We also performed a reanalysis and meta-analysis of published data and found similar results. In some cases, the dose–response was similar to IAH, whereas in others, it was more gradual. Although the IAH model was supported for some individual data sets, overall, the more complex DA and HHS models were better supported. Thus, we can conclude that the dose–response kinetics of WSSV in shrimp appear to be variable over experiments, and that in many cases, the IAH model is not suitable for the analysis of WSSV dose–response. We do not think that virus amplification would have affected the model selection procedure (see Supporting Information Text S1).

Given that neither the DA nor the HHS models are consistently better supported in both analyses presented here, it is difficult to draw any firm conclusions on what mechanisms might underlie the deviations from the IAH model observed. However, by considering the mechanisms that underlie each model and their variable occurrence over experiments, we can attempt to reach some tentative conclusion on which model appears to be more reasonable. Under the DA model, the gradual dose–response we have observed is indicative of antagonistic dose-dependent interactions, as  $\kappa < 1$ . Whilst there are striking examples of synergistic dose-dependent action (Borges, Zhang, Rollins, Osuna, Wiedenheft, & Bondy-Denomy, In press; Fulton, 1962; Landsberger et al., 2018; Sánchez-Navarro et al., 2013), to the best of our knowledge, antagonistic

interactions during coinoculation have not been observed for other viruses (Zwart & Elena, 2015). Thus, it is not clear what underlying mechanism would cause inconsistent antagonistic interactions within a conspecific virus population, although one possibility is dose-dependent priming of the immune system.

Whereas the probability that a virus particle causes host infection is fixed for the IAH model, it follows a probability distribution over hosts for the HHS model. The HHS model, although being more complex than the IAH model, therefore does not violate the principle of independent action whilst predicting a more gradual dose–response (Regoes et al., 2003; van der Werf et al., 2011). Consequently, if the distribution of susceptibility is different for the cohorts of shrimp used for different experiments, we could reasonably expect different shapes for the dose–response in different experiments, and support for the IAH or HHS model depending on how much variation in the distribution of susceptibility is present in the shrimp population. The shrimp populations used in our two experiments differed in age and mean weight, although they were taken from the same cohort and have a similar standard deviation for weight. Likewise, the data sets included in the reanalysis represent experiments that were performed independently, implying that different amounts of variation in susceptibility is entirely plausible. For instance, all nine data sets reported in one study (Laramore et al., 2009) were carried out with different cohorts of shrimp. There can be large differences in host susceptibility to a pathogen (Ben-Ami, Ebert, & Regoes, 2010; Chakrabarty, Dutta, Mallik, Mondal, & Mandal, 2015) and for insects susceptibility can be phenotypically





**FIGURE 3** Logit  $R^2$  against log host weight for the independent action hypothesis (IAH) model (panel a), dose-dependent action (DA) model (panel b), and heterogeneous host susceptibility (HHS) model (panel c). Black lines correspond to fitted values from random effects meta-regression (REMR), only that in 1a is statistically significant (Supporting Information Table S3). In Panels d–f, logit  $R^2$  values are plotted against host species for the IAH, DA and HHS models. Black points and error bars (95% confidence intervals) correspond to overall effects for each species as estimated by REMR (differences among species were not statistically significant; Supporting Information Table S3). Grey points are effect sizes, with size scaled by precision

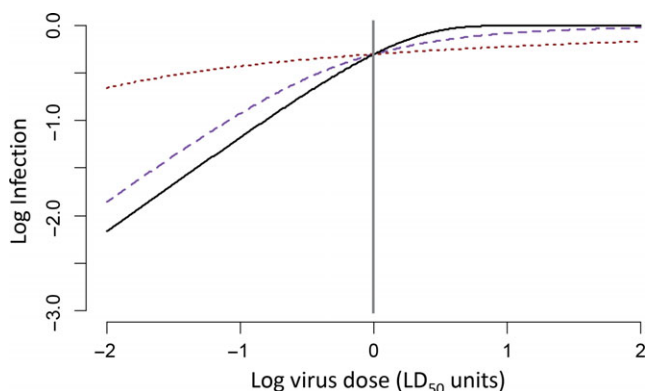
plastic and linked to environmental factors such as host density (Reeson, Wilson, Gunn, Hails, & Goulson, 1998). Although we cannot rule out antagonistic dose-dependent action, we therefore think that the most likely explanation for deviations from IAH model predictions is differences in the distribution of host susceptibility to WSSV. The meta-analysis results also suggest that the IAH model fits better when shrimp with a higher weight are used, raising the possibility that heterogeneous susceptibility to WSSV might decrease as development progresses. This contrasts with observations for baculovirus infection of insect larvae, in which the IAH model was supported early and not late in larval development (Zwart et al., 2009), and we speculate these differences might arise due to differences in the timing of investment in viral defences.

Similar to previous but shorter-running experiments (Escobedo-Bonilla et al., 2005), infection was mainly PCR detected in shrimp that had died by the end of the experiment. Some of these surviving shrimp may represent true sublethal infections (Johnson et al., 2008; Venegas et al., 2000; Wu & Muroga, 2004). These shrimp could also have become infected later in the experiment due to waterborne transmission, in which case death could have been delayed simply due to later

onset of infection. Our data suggest that—for the conditions we have studied—sublethal infections will not have a high prevalence at any virus-particle dose. For all models and data sets, we estimated large values for the probability of host death upon virus infection ( $\varphi > 0.5$ ). On the other hand, these results are also puzzling because WSSV infection is often detected in shrimp sampled from ponds in which there are no indications of a disease outbreak (Flegel et al., 2004; Hoa, Zwart, Phuong, Oanh, et al., 2011). These results suggest that the timing of infection, infection route or environmental conditions found in the field strongly affect WSSV virulence. Alternatively, the kinetics of infection and mortality for the WSSV isolates used in our experiments may not be representative for strains circulating on farms. The virus isolate used here was sampled during incipient epizootics and might be more representative of high-virulence strains that perform well under these outbreak conditions.

For the studies included in the meta-analysis, 13 different virus isolates were used (Supporting Information Table S2). Most of these studies report only infection or mortality, so we could not estimate the probability of death upon infection and hereby determine the generality of our result that this probability is large. One interesting related

question is whether virus isolate (and by extension virus genotype) affected the observed infection kinetics. Although there were insufficient studies with the same virus isolate to allow for a formal analysis of whether virus genotype affects  $R^2$ , two isolates were used in three different studies (TH-96 and Söderhall TH-96; see Supporting Information Table S2). In each study, a different model had the highest  $R^2$ , suggesting that other factors affect model selection more strongly than virus isolate, and by implication that virus genotype does not affect the type of infection kinetics. Note that we do expect to see different infection probabilities for different virus isolates: The position of the dose–response will shift, as some virus genotypes will infect more easily (see also Supporting Information Figure S1), and some shrimp populations will be more susceptible. By contrast, we expect the degree of HHS within the shrimp population to be a more important determinant of the type of infection kinetics (i.e., the shape of the dose–response). An epidemiological model that would include all the main factors we have found to affect rates of infection would therefore allow for mass action, but also incorporate (a) virus strains with different infection probabilities; and (b) shrimp populations with different degrees of HHS.



**FIGURE 4** The predicted effects of heterogeneity in host susceptibility on the rate of infection are shown. The ordinate is  $\log_{10}$  of virus dose in median lethal dose ( $LD_{50}$ ) units, whilst the abscissae are the  $\log_{10}$ -transformed rate of infection. The vertical grey line indicates one  $LD_{50}$  and using the  $LD_{50}$  scale allows us to compare the predicted rate of infection for different models compared to a common, convenient measure of infectivity. The solid black line is the dose–response predicted by the independent action hypothesis (IAH) model. The dashed purple line represents the heterogeneous host susceptibility (HHS) model prediction with the same variation in host susceptibility as estimated for experiment #2 ( $\nu = 1.905$ ), whereas the dotted dark red line represents the HHS model prediction based on the second highest level of variation predicted for data sets in the reanalysis of published data ( $\nu = 10.233$ , based on the seventh data set in Supporting Information Table S2; white spot syndrome virus CH-1995-2). The IAH model will underestimate the rate of infection for virus dose below the equivalent of an  $LD_{50}$ , whilst overestimating infection for higher doses. The underestimation of the rate of infection for low doses is probably more relevant for risk assessment because the infection and death of one or more highly susceptible individuals will lead to all individuals in the pond being exposed to higher doses

Finally, our results here have two practical implications. First, both the HHS and the DA models can predict a more gradual dose–response than the IAH model, which has implications for risk analysis. The IAH model can underestimate the frequency of infection at low doses when there is host heterogeneity (Teunis & Havelaar, 2000), or overestimate it when there are synergistic dose-dependent interactions (Cornforth, Matthews, Brown, & Raymond, 2015), highlighting the importance of using empirically supported infection models for assessments. Our results also suggest that infection will be underestimated by the IAH model for low WSSV doses, given the amount of heterogeneity in the host population estimated for experiment #2 and an extreme example from the data reanalysis (Figure 4). Second, consideration of heterogeneity of host susceptibility is indispensable to model and understand infectious disease dynamics. Although individuals with a low susceptibility could facilitate between-pond or between-herd transmission by virtue of being susceptible to low pathogen doses often associated with long-range transmission, in general, heterogeneity makes it harder for a pathogen to spread within a population (Cook, Otten, Marion, Gibson, & Gilligan, 2007; Dwyer, Elkinton, & Buonaccorsi, 1997). Our results therefore suggest that the effects of heterogeneous susceptibility are relevant to understanding WSSV infection and transmission in shrimp, whilst at the same time suggesting that such heterogeneity is not present in all shrimp cohorts.

## ACKNOWLEDGEMENTS

The authors thank Sietze Leenstra (Aquaculture Research Facility of Wageningen University and Research) for his invaluable assistance with shrimp rearing, Thomas Jung of von Wussow Importe GmbH for assistance with shrimp import, Gold Coin Singapore (formerly Syaqua) for assistance with the shrimp and feed, Dr. Bui Thi Minh Dieu (Can Tho University, Vietnam) for help with setting up the PCR assays, and Prof. Dr. Mart de Jong (Wageningen University and Research) for useful discussions.

## ORCID

Mark P. Zwart  <http://orcid.org/0000-0003-4361-7636>

## REFERENCES

- Ben-Ami, F., Ebert, D., & Regoes, R. R. (2010). Pathogen dose infectivity curves as a method to analyze the distribution of host susceptibility: A quantitative assessment of maternal effects after food stress and pathogen exposure. *The American Naturalist*, 175, 106–115. <https://doi.org/10.1086/648672>
- Ben-Ami, F., Regoes, R. R., & Ebert, D. (2008). A quantitative test of the relationship between parasite dose and infection probability across different host-parasite combinations. *Proceedings of the Royal Society B: Biological Sciences*, 275, 853–859. <https://doi.org/10.1098/rspb.2007.1544>
- Borges, A. L., Zhang, J. Y., Rollins, M. F., Osuna, B. A., Wiedenheft, B., & Bondy-Denomy, J. (In press). Bacteriophage cooperation suppresses

- CRISPR-Cas3 and Cas9 immunity. *Cell*. <https://doi.org/10.1016/j.cell.2018.06.013>
- Chakrabarty, U., Dutta, S., Mallik, A., Mondal, D., & Mandal, N. (2015). Identification and characterisation of microsatellite DNA markers in order to recognise the WSSV susceptible populations of marine giant black tiger shrimp, *Penaeus monodon*. *Veterinary Research*, 1–10. <https://doi.org/10.1186/s13567-015-0248-2>
- Cook, A. R., Otten, W., Marion, G., Gibson, G. J., & Gilligan, C. A. (2007). Estimation of multiple transmission rates for epidemics in heterogeneous populations. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 20392–20397. <https://doi.org/10.1073/pnas.0706461104>
- Cornforth, D. M., Matthews, A., Brown, S. P., & Raymond, B. (2015). Errors in pathogen risk assessment due to the failure of the independent action hypothesis. *PLOS Pathogens*, 11, e1004775. <https://doi.org/10.1371/journal.ppat.1004775>
- Dieu, B. T. M., Marks, H., Siebenga, J. J., Goldbach, R. W., Zuidema, D., Duong, T. P., & Vlak, J. M. (2004). Molecular epidemiology of white spot syndrome virus within Vietnam. *The Journal of General Virology*, 85, 3607–3618. <https://doi.org/10.1099/vir.0.80344-0>
- Dieu, B. T. M., Zwart, M. P., & Vlak, J. M. (2010). Can VNTRs be used to study genetic variation within white spot syndrome virus isolates? *Journal of Fish Diseases*, 33, 689–693. <https://doi.org/10.1111/j.1365-2761.2010.01163.x>
- Druett, H. A. (1952). Bacterial invasion. *Nature*, 170, 288. <https://doi.org/10.1038/170288a0>
- Dwyer, G., Elkinton, J. S., & Buonaccorsi, J. P. (1997). Host heterogeneity in susceptibility and disease dynamics: Tests of a mathematical model. *The American Naturalist*, 150, 685–707. <https://doi.org/10.1086/286089>
- Escobedo-Bonilla, C. M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M. B., & Nauwynck, H. J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *Journal of Fish Diseases*, 31, 1–18. <https://doi.org/10.1111/j.1365-2761.2007.00877.x>
- Escobedo-Bonilla, C. M., Wille, M., Sanz, V. A., Sorgeloos, P., Pensaert, M. B., & Nauwynck, H. J. (2005). In vivo titration of white spot syndrome virus (WSSV) in specific pathogen-free *Litopenaeus vannamei* by intramuscular and oral routes. *Diseases of Aquatic Organisms*, 66, 163–170. <https://doi.org/10.3354/dao066163>
- Flegel, T. W. (2007). Update on viral accommodation, a model for host-viral interaction in shrimp and other arthropods. *Developmental and Comparative Immunology*, 31, 217–231. <https://doi.org/10.1016/j.dci.2006.06.009>
- Flegel, T. W., Nielsen, L., Thamavit, V., Kongtim, S., & Pasharawipas, T. (2004). Presence of multiple viruses in non-diseased, cultivated shrimp at harvest. *Aquaculture*, 240, 55–68. <https://doi.org/10.1016/j.aquaculture.2004.06.032>
- Fulton, R. W. (1962). The effect of dilution on Necrotic ring spot virus infectivity and the enhancement of infectivity by noninfective virus. *Virology*, 18, 477–485.
- Hoa, T. T. T., Zwart, M. P., Phuong, N. T., de Jong, M. C. M., & Vlak, J. M. (2012). Low numbers of repeat units in variable number of tandem repeats (VNTR) regions of white spot syndrome virus are correlated with disease outbreaks. *Journal of Fish Diseases*, 35, 817–826. <https://doi.org/10.1111/j.1365-2761.2012.01406.x>
- Hoa, T. T. T., Zwart, M. P., Phuong, N. T., Oanh, D. T. H., de Jong, M. C. M., & Vlak, J. M. (2011). Mixed-genotype white spot syndrome virus infections of shrimp are inversely correlated with disease outbreaks in ponds. *Journal of General Virology*, 92, <https://doi.org/10.1099/vir.0.026351-0>
- Hoa, T. T. T., Zwart, M. P., Phuong, N. T., Vlak, J. M., & de Jong, M. C. M. (2011). Transmission of white spot syndrome virus in improved-extensive and semi-intensive shrimp production systems: A molecular epidemiology study. *Aquaculture*, 313, 7–14. <https://doi.org/10.1016/j.aquaculture.2011.01.013>
- van Hulst, M. C. W., Witteveldt, J., Peters, S., Kloosterboer, N., Tarchini, R., Fiers, M., ... Vlak, J. M. (2001). The white spot syndrome virus DNA genome sequence. *Virology*, 286, 7–22. <https://doi.org/10.1006/viro.2001.1002>
- van Hulst, M. C., Witteveldt, J., Snippe, M., & Vlak, J. M. (2001). White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. *Virology*, 233, 228–233. <https://doi.org/10.1006/viro.2001.0928>
- Johnson, J. B., & Omland, K. S. (2004). Model selection in ecology and evolution. *Trends in Ecology & Evolution*, 19, 101–108. <https://doi.org/10.1016/j.tree.2003.10.013>
- Johnson, K. N., van Hulst, M. C. W., & Barnes, A. C. (2008). “Vaccination” of shrimp against viral pathogens: Phenomenology and underlying mechanisms. *Vaccine*, 26, 4885–4892. <https://doi.org/10.1016/j.vaccine.2008.07.019>
- Ladner, J. T., Wiley, M. R., Beitzel, B., Kramer, L. D., Tesh, R. B., Palacios, G., ... Weaver, S. C. (2016). A multicomponent animal virus isolated from mosquitoes. *Cell Host & Microbe*, 20, 1–11. <https://doi.org/10.1016/j.chom.2016.07.011>
- Landsberger, M., Gandon, S., Meaden, S., Rollie, C., Chevillereau, A., Chabas, H., Buckling, A., Westra, E. R., & vanHoute, S. (In press). Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity. *Cell*. <https://doi.org/10.1016/j.cell.2018.05.058>
- Laramore, S. E., Scarpa, J., Laramore, C. R., & Lin, J. (2009). Virulence variation of white spot syndrome virus in Pacific white shrimp *Litopenaeus vannamei*. *Journal of Aquatic Animal Health*, 21, 82–90. <https://doi.org/10.1577/H08-012.1>
- Marks, H., van Duijse, J. J. A., Zuidema, D., van Hulst, M. C. W., & Vlak, J. M. (2005). Fitness and virulence of an ancestral white spot syndrome virus isolate from shrimp. *Virus Research*, 110, 9–20. <https://doi.org/10.1016/j.virusres.2005.01.002>
- McFadden, D. (1973). Conditional logit analysis of qualitative choice behavior. In P. Zarembka (Ed.), *Frontiers in econometrics* (pp. 105–142). Cambridge, MA: Academic Press.
- Mengersen, K., & Gurevitch, J. (2013). Using other metrics of effect size in meta-analysis. In J. Koricheva, J. Gurevitch, & K. Mengersen (Eds.), *Handbook of meta-analysis in ecology and evolution* (p. 592). Princeton, NJ: Princeton University Press. <https://doi.org/10.23943/princeton/9780691137285.001.0001>
- Motamedi Sedeh, F., Afsharnasab, M., Heidareh, M., Shafae, S. K., Rajabifar, S., Dashtianasab, A., & Razavi, M. H. (2012). Titration of the Iranian White Spot Virus isolate, on Crayfish *Astacus leptodactylus* and *Penaeus semisulcatus*. *Iranian Journal of Fisheries Sciences*, 11, 145–155.
- Nakagawa, S., Noble, D. W. A., Senior, A. M., & Lagisz, M. (2017). Meta-evaluation of meta-analysis: Ten appraisal questions for biologists. *BMC Biology*, 15, 1–14. <https://doi.org/10.1186/s12915-017-0357-7>
- Nakagawa, S., & Santos, E. S. A. (2012). Methodological issues and advances in biological meta-analysis. *Evolutionary Ecology*, 26, 1253–1274. <https://doi.org/10.1007/s10682-012-9555-5>
- Navakatikyan, M. (2007). A model for residence time in concurrent variable interval performance. *Journal of the Experimental Analysis of Behaviour*, 87, 121–141.
- Phuoc, L. H., Corteel, M., Nauwynck, H. J., Pensaert, M. B., Alday-Sanz, V., van den Broeck, W., ... Bossier, P. (2008). Increased susceptibility of white spot syndrome virus-infected *Litopenaeus vannamei* to *Vibrio campbellii*. *Environmental Microbiology*, 10, 2718–2727. <https://doi.org/10.1111/j.1462-2920.2008.01692.x>
- Pradeep, B., Karunasagar, I., & Karunasagar, I. (2009). Fitness and virulence of different strains of white spot syndrome virus. *Journal of Fish Diseases*, 32, 801–805. <https://doi.org/10.1111/j.1365-2761.2009.01053.x>
- Prior, S., Browdy, C. L., Shepard, E. F., Laramore, R., & Parnell, P. G. (2003). Controlled bioassay systems for determination of lethal infective doses of tissue homogenates containing Taura syndrome or

- white spot syndrome virus. *Diseases of Aquatic Organisms*, 54, 89–96. <https://doi.org/10.3354/dao054089>
- R Core Team (2016). *R: A language and environment for statistical computing*. Vienna, Austria.
- Rahman, M. M., Corteel, M., Dantas-Lima, J. J., Wille, M., Alday-Sanz, V., Pensaert, M. B., ... Nauwynck, H. J. (2007). Impact of daily fluctuations of optimum (27°C) and high water temperature (33°C) on *Penaeus vannamei* juveniles infected with white spot syndrome virus (WSSV). *Aquaculture*, 269, 107–113. <https://doi.org/10.1016/j.aquaculture.2007.04.056>
- Rahman, M. M., Corteel, M., Escobedo-Bonilla, C. M., Wille, M., Alday-Sanz, V., Pensaert, M. B., ... Nauwynck, H. J. (2008). Virulence of white spot syndrome virus (WSSV) isolates may be correlated with the degree of replication in gills of *Penaeus vannamei* juveniles. *Diseases of Aquatic Organisms*, 79, 191–198. <https://doi.org/10.3354/dao01907>
- Rahman, M. M., Escobedo-Bonilla, C. M., Corteel, M., Dantas-Lima, J. J., Wille, M., Sanz, V. A., ... 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. <https://doi.org/10.1016/j.aquaculture.2006.09.007>
- Reeson, A. F., Wilson, K., Gunn, A., Hails, R. S., & Goulson, D. (1998). Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proceedings of the Royal Society B: Biological Sciences*, 265, 1787–1792. <https://doi.org/10.1098/rspb.1998.0503>
- Regoes, R. R., Hottinger, J. W., Sygnarski, L., & Ebert, D. (2003). The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle. *Epidemiology and Infection*, 131, 957–966.
- Sánchez-Navarro, J.-A., Zwart, M. P., & Elena, S. F. (2013). Effects of the number of genome segments on primary and systemic infections with a multipartite plant RNA virus. *Journal of Virology*, 87, 10805–10815. <https://doi.org/10.1128/JVI.01402-13>
- Soto, M. A., & Lotz, J. M. (2001). Epidemiological parameters of white spot syndrome virus infections in *Litopenaeus vannamei* and *L. setiferus*. *Journal of Invertebrate Pathology*, 15, 9–15. <https://doi.org/10.1006/jipa.2001.5035>
- Teunis, P. F. M., & Havelaar, A. H. (2000). The beta poisson dose-response model is not a single-hit model. *Risk Analysis*, 20, 513–520. <https://doi.org/10.1111/0272-4332.204048>
- Thuong, K., Tuan, V. V., Li, W., Sorgeloos, P., Bossier, P., & Nauwynck, H. (2016). Per os infectivity of white spot syndrome virus (WSSV) in white-legged shrimp (*Litopenaeus vannamei*) and role of peritrophic membrane. *Veterinary Research*, 1–12. <https://doi.org/10.1186/s13567-016-0321-5>
- Venegas, C. A., Nonaka, L., Mushiake, K., Nishizawa, T., & Muroga, K. (2000). Quasi-immune response of *Penaeus japonicus* to penaeid rod-shaped DNA virus (PRDV). *Diseases of Aquatic Organisms*, 42, 83–89. <https://doi.org/10.3354/dao042083>
- Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36, 1–48. <https://doi.org/10.18637/jss.v036.i03>
- Waikhom, G., John, K. R., George, M. R., & Jeyaseelan, M. J. P. (2006). Differential host passaging alters pathogenicity and induces genomic variation in white spot syndrome virus. *Aquaculture*, 261, 54–63. <https://doi.org/10.1016/j.aquaculture.2006.07.031>
- Walker, P. J., Gudkovs, N., Pradeep, B., Stalin Raj, V., Sergeant, E., Chandra Mohan, A. B., ... Mohan, C. V. (2011). Longitudinal disease studies in small-holder black tiger shrimp (*Penaeus monodon*) farms in Andhra Pradesh, India. III. A complex dynamic of WSSV infection and WSSV genotype distribution in farmed shrimp and wild crustaceans. *Aquaculture*, 319, 319–327. <https://doi.org/10.1016/j.aquaculture.2011.07.035>
- Walker, P. J., & Mohan, C. V. (2009). Viral disease emergence in shrimp aquaculture: Origins, impact and the effectiveness of health management strategies. *Reviews in Aquaculture*, 1, 125–154. <https://doi.org/10.1111/j.1753-5131.2009.01007.x>
- Warton, D. I., & Hui, F. K. C. (2011). The arcsine is asinine: The analysis of proportions in ecology. *Ecology*, 92, 3–10. <https://doi.org/10.1890/10-0340.1>
- van der Werf, W., Hemerik, L., Vlak, J. M., & Zwart, M. P. (2011). Heterogeneous host susceptibility enhances prevalence of mixed-genotype micro-parasite infections. *PLoS computational biology*, 7, e1002097. <https://doi.org/10.1371/journal.pcbi.1002097>
- Witteveldt, J., Cifuentes, C. C., Vlak, J. M., & van Hulten, M. C. W. (2004). Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. *Journal of Virology*, 78, 2057–2061. <https://doi.org/10.1128/JVI.78.4.2057-2061.2004>
- Xie, X., Li, H., Xu, L., & Yang, F. (2005). A simple and efficient method for purification of intact white spot syndrome virus (WSSV) viral particles. *Virus Research*, 108, 63–67. <https://doi.org/10.1016/j.virusres.2004.08.002>
- Yang, F., He, J., Lin, X., Li, Q., Pan, D., & Xu, X. U. N. (2001). Complete genome sequence of the shrimp white spot bacilliform virus. *Journal of Virology*, 75, 11811–11820. <https://doi.org/10.1128/JVI.75.23.11811-11820.2001>
- Wu, J. L., & Muroga, K. (2004). Apoptosis does not play an important role in the resistance of 'immune' *Penaeus japonicus* against white spot syndrome virus. *Journal of Fish Diseases*, 2, 15–21. <https://doi.org/10.1046/j.1365-2761.2003.00491.x>
- Zwart, M. P., Daròs, J.-A., & Elena, S. F. (2011). One is enough: In vivo effective population size is dose-dependent for a plant RNA virus. *PLoS pathogens*, 7, e1002122. <https://doi.org/10.1371/journal.ppat.1002122>
- Zwart, M. P., Dieu, B. T. M., Hemerik, L., & Vlak, J. M. (2010). Evolutionary trajectory of white spot syndrome virus (WSSV) genome shrinkage during spread in Asia. *PLoS One*, 5, e13400. <https://doi.org/10.1371/journal.pone.0013400>
- Zwart, M. P., & Elena, S. F. (2015). Matters of size: Genetic bottlenecks in virus infection and their potential impact on evolution. *Annual Review of Virology*, 2, 161–179. <https://doi.org/10.1146/annurev-virol-100114-055135>
- Zwart, M. P., Hemerik, L., Cory, J. S., de Visser, J. A. G. M., Bianchi, F. J. A. van Oers, M. M., ... van der Werf, W. (2009). An experimental test of the independent action hypothesis in virus-insect pathosystems. *Proceedings of the Royal Society B: Biological Sciences*, 276, 2233–2242. <https://doi.org/10.1098/rspb.2009.0064>

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Ngo TTN, Senior AM, Culina A, Santos ESA, Vlak JM, Zwart MP. Quantitative analysis of the dose–response of white spot syndrome virus in shrimp. *J Fish Dis*. 2018;00:1–12. <https://doi.org/10.1111/jfd.12877>