

# Transmission of white spot syndrome virus (WSSV) to *Litopenaeus vannamei* from infected cephalothorax, abdomen, or whole shrimp cadaver

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**ABSTRACT:** Shrimp viruses can remain infectious in frozen shrimp tissue and have been found in frozen commodity shrimp. Therefore, the threat of viral outbreaks in wild and cultured shrimp via frozen commodity shrimp exists. Because frozen shrimp are imported with and without the cephalothorax, more knowledge is needed concerning the infectivity of a cephalothorax relative to that of an abdomen. We compared the mortality rates from shrimp exposed to a WSSV-infected cephalothorax, abdomen, or whole shrimp cadaver. Estimates of transmission coefficients from the exposures to the infected cephalothorax, abdomen, or whole shrimp were also calculated because the transmission coefficients account for differences in the initial doses. In addition, we compared the variability in infectivity of pieces of shrimp by feeding 24 equal-sized pieces of cephalothorax and abdomen to 24 individually isolated shrimp. In Expt 1, susceptible shrimp did not completely consume the infected abdomen, and a significant difference was detected among shrimp exposed to the abdomen (mortality rate = 0.40), cephalothorax (mortality rate = 0.75), and whole shrimp cadaver (mortality rate = 0.67). The calculated transmission coefficients were 0.95 from an infected cephalothorax, 0.59 from an infected abdomen, and 0.69 from an infected whole shrimp cadaver. In Expt 2, susceptible shrimp were starved to ensure complete ingestion of each dose. No significant difference was observed in the estimated mortality rates from an infected cephalothorax (0.58), abdomen (0.63), or whole shrimp (0.67). The calculated transmission coefficients were 0.84 from an infected cephalothorax, 0.83 from an infected abdomen, and 0.60 from an infected whole shrimp cadaver. In Expt 3, no difference was observed in the mortality rates resulting from exposures to pieces of infected cephalothorax (0.57) or abdomen (0.58). Our results suggested that there was no difference in the viral loads of a WSSV-infected cephalothorax or abdomen, but that the cephalothorax was more infectious, probably because it was more palatable. In addition, our results are inconsistent with some assumptions of pathogen transmission used in epidemiological models. Some shrimp may be less aggressive feeders; therefore, susceptible shrimp are differentially contacting the dead infected shrimp in the exposure tanks, violating the random mixing assumption. Moreover, virus is probably not homogeneously distributed throughout an infected shrimp, suggesting that contacts between susceptible and infected shrimp are not equally likely to result in transmission.

**KEY WORDS:** Mass action · Epidemiology models · Shrimp virus · Frozen commodity shrimp

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## INTRODUCTION

Importation of commodity shrimp into the US has increased substantially within the last 2 decades with

much coming from shrimp farming. During this period about 20 shrimp viruses have been reported (Lightner 1996). Some of these viruses have been found to be infectious in frozen commodity shrimp (Nunan et al. 1998), suggesting that frozen shrimp tissue may be a mechanism for spread (Eastern Research Group 1999).

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Historically, shrimp processing plants in the US have functioned seasonally, primarily handling the domestic fisheries catch, and so are often located along the coast near the shrimp fleet. Today, many processing plants function year-round, handling frozen imported shrimp when the domestic, wild harvest is low. The solid and liquid waste from processing imported shrimp may contain infectious virus.

Most of the shrimp viruses infect many species of penaeid shrimp (Lightner 1996, Lightner et al. 1998), and some like white spot syndrome virus (WSSV) have additional non-penaeid decapod hosts (Supamattaya et al. 1998). Several potential hosts of WSSV support important fisheries in the US, and these species utilize coastal estuaries as nursery grounds. Therefore, the possibility exists that these coastal inhabitants may contact virus in processing plant liquid or solid waste.

Frozen commodity shrimp may pose a threat to farmed shrimp and to some shrimp and crab fisheries. To further understand the threat, more knowledge concerning the infectivity of frozen shrimp is needed. For example, there may be a difference in the infectivity between the cephalothorax and the abdomen of shrimp. Because frozen shrimp are imported with and without the cephalothorax, we undertook a study to compare the infectivity of the cephalothorax, the abdomen, and the whole shrimp cadaver.

We compared the infectivity of the cephalothorax, abdomen and whole shrimp by estimating mortality and transmission coefficients. The mortality is the proportion of shrimp dying from each type of exposure: cephalothorax, abdomen, or whole shrimp cadaver. Mortality determined this way does not consider the differences in dose from each exposure type. The whole cadaver is about twice the dose of either of the other 2 types. The differences in dose can be accounted for by estimating transmission based on epidemiological models.

We estimated transmission coefficients by using an equation that is derived from a standard epidemiological model of the Reed-Frost type (Abbey 1952). The dynamics of pathogen transmission from this model can be represented as such:

$$S_1 = S_0 - S_0[1 - (1 - \beta)^{I_0}] \quad (1)$$

where  $S_0$  and  $I_0$  are the number of susceptible and infected hosts, respectively, at time = 0,  $S_1$  is the number of susceptible hosts remaining at some later time  $t = 1$ , and  $\beta$  is the transmission coefficient (the probability that a contact between susceptible and an infected host will result in a transmission). By solving for  $\beta$  in Eq. (1), an estimate of the transmission coefficient can be obtained (Lotz & Soto unpubl., Soto & Lotz in press),

$$\beta = 1 - \exp\left(\frac{\ln \frac{S_1}{S_0}}{I_0}\right) \quad (2)$$

If  $I_0$  is taken as 0.5 for each of the cephalothorax and abdomen exposures, and  $I_0$  is taken as 1 for a whole shrimp cadaver exposure, the transmission coefficient can be standardized for different doses.

There are at least 3 assumptions for estimating the transmission coefficient ( $\beta$ ) this way (Dwyer 1997). The first is that each susceptible host must have an equal chance of contacting an infected shrimp, the random mixing assumption. The second is that each contact between a susceptible and an infected host is as likely to pass infection as another, in other words, that each individual is equally susceptible to a pathogen and that all infected individuals are equally infectious. For pathogens that are transmitted primarily by ingestion of infected cadavers, such as WSSV (Soto & Lotz in press), virus must be homogeneously distributed throughout the body and each portion must be equally palatable because shrimp acquire infections from ingesting portions of an infected shrimp. A third assumption is that  $\beta$  is not affected by density.

The objectives of the 3 experiments conducted in this study were 2-fold. The first objective was to compare the relative infectivity of WSSV-infected cephalothoraces, abdomens, and whole shrimp cadavers. The second objective was to test assumptions regarding the method of pathogen transmission used in epidemiology models. Specifically, we tested if random mixing of susceptible and infected hosts occurs for WSSV and *Litopenaeus vannamei* (formerly called *Penaeus vannamei*) if each contact between susceptible and infected hosts is as likely to pass infection as any other, and if  $\beta$  is affected by density.

In Expts 1 and 2, we used Eq. (2) to estimate the transmission coefficient for susceptible shrimp ( $S_0$ ) from an infected cephalothorax ( $I_0 = 0.5$ ), abdomen ( $I_0 = 0.5$ ), or whole shrimp ( $I_0 = 1$ ). If the random mixing assumption is met, the total transmission resulting from an infected cephalothorax and abdomen should equal the transmission resulting from an infected whole shrimp. In addition, if virus is distributed equally between the cephalothorax and abdomen and each part is equally palatable, there should be as much transmission resulting from ingestion of the cephalothorax as from ingestion of the abdomen because the cephalothorax and abdomen weigh approximately the same (see 'Results').

Expt 3 was conducted to determine if each contact between susceptible and infected hosts is as likely to pass infection as another. An infected shrimp was dissected into 24 equally sized pieces, 12 pieces from the cephalothorax and 12 from the abdomen. Each piece was then fed to 24 individually isolated shrimp. If each

shrimp is equally susceptible to WSSV, if virus is homogeneously distributed throughout the body, and if each piece is equally palatable, then 24 infections should arise.

## MATERIALS AND METHODS

**Test animals and viral stock.** Shrimp used in these experiments were from the original unselected population of shrimp (*Litopenaeus vannamei*, Kona stock) that have been maintained by the United States Marine Shrimp Farming Program at the Oceanic Institute in Hawaii (Lotz 1997). All shrimp used weighed between 1 and 3 g. The isolate of WSSV used was obtained from mainland China in 1998 and has been maintained in *L. vannamei* since then. The WSSV used in this study had been passed 9 times through *L. vannamei*.

**Estimating the transmission coefficient.** The transmission coefficient was calculated as in Eq. (2), using the initial numbers of susceptible ( $S_0$ ) and infected ( $I_0$ ) shrimp and the number of susceptible shrimp ( $S_1$ ) at the end of a time period of interest. Experimentally, 12 susceptible shrimp were exposed to an infected shrimp for a specified period of time, then the exposed shrimp were isolated individually for any infections to become patent, and finally all exposed shrimp were examined histologically to determine infection status (Lotz & Soto unpubl., Soto & Lotz in press).

**Expts 1 and 2. Infectivity from cephalothorax, abdomen, or whole shrimp.** The experimental procedure for obtaining the transmission coefficient estimates was divided into 4 phases: preparation of  $I_0$  (initial infected shrimp), exposure, isolation, and diagnosis (Soto & Lotz in press).

**$I_0$  preparation:** To prepare the initial infected shrimp used for exposure ( $I_0$ ), we injected 200 *Litopenaeus vannamei* with a cell-free shrimp homogenate containing WSSV. The homogenate consisted of a 1:10 (w/v) dilution of tissue from shrimp known to have died of WSSV with distilled water. Each shrimp was injected with 0.02 ml g<sup>-1</sup> body weight into the muscle of the third abdominal segment. Shrimp displayed signs of disease at 24 h post-exposure and moribund shrimp were immediately placed in a -70°C freezer.

**Exposure:** In the exposure phase, susceptible shrimp ( $S_0$ ) were exposed to infected shrimp ( $I_0$ ). Twelve susceptible shrimp and 1 infected cephalothorax, abdomen, or whole shrimp cadaver were placed in a cylindrical tank (1 m<sup>2</sup> surface area by 0.6 m height). For the cephalothorax and abdomen exposures, a whole infected shrimp was bisected immediately posterior to the cephalothorax. The weight to the nearest 0.1 g of the cephalothorax, abdomen, or whole shrimp was taken prior to and after exposure. Each exposure type was replicated 4 times for a total of 12 tanks. Twelve

additional unexposed shrimp in a single tank served as the negative control and were fed commercially available shrimp food. Each tank was filled to a depth of 10 cm with chlorine treated seawater. The susceptible shrimp were exposed to the infected material for 24 h. Water temperature in the transmission tanks was maintained at 26 ± 1°C.

In Expt 1, susceptible shrimp were not starved, whereas in Expt 2, susceptible shrimp and negative control shrimp were starved for 3 d prior to exposures to ensure complete ingestion of the infected material.

**Isolation:** To ensure no secondary transmission, the exposed susceptible shrimp were isolated after the 24 h exposure period into 1 l jars. All jars were placed in a water bath, and each jar was supplied with an air line. Water temperature in the bath was maintained at 26 ± 1°C. The time of death of isolated shrimp was recorded. Shrimp that died during the isolation phase were fixed in Davidson's solution following procedures outlined by Lightner (1996). Shrimp were kept in these isolation jars for 5 d, after which all surviving specimens were similarly fixed.

**Diagnosis:** To determine the number of susceptible shrimp remaining after exposure ( $S_1$ ), shrimp were examined histologically (Hematoxylin and Eosin stains) for the presence of WSSV intranuclear inclusions. The 12 negative control shrimp were also examined. Two non-serial, sagittal sections of each shrimp were analyzed. Previously we found that 100% of shrimp dying during the isolation phase were WSSV positive, and that 98.1% of all shrimp surviving the isolation phase were histologically negative for WSSV (Soto & Lotz in press). Therefore, in this study, a sample of shrimp was examined to determine if the same pattern existed. All shrimp from 1 tank in each of the cephalothorax, abdomen, and whole shrimp exposures were examined histologically for the presence of WSSV intranuclear inclusions. In addition, the 12 negative control animals were examined for WSSV inclusions.

**Expt 3. Infectivity from pieces of cephalothorax and abdomen.** In Expts 1 and 2, 12 susceptible shrimp were exposed to an infected cephalothorax, abdomen, or whole shrimp in 1 m<sup>2</sup> tanks (bottom surface area). In Expt 3, the cephalothorax and abdomen were dissected into 12 pieces of approximately equal size, and 12 susceptible shrimp were isolated individually in 1 l jars and exposed to 1 piece of infected tissue from the cephalothorax or abdomen. The dissections were as follows: a whole infected shrimp was bisected immediately posterior to the cephalothorax, the cephalothorax and abdomen were then cut mid-sagittally, then each quarter was cut into 6 pieces, with each cut being made perpendicular to the sagittal plane. The weight to the nearest 0.1 g of the cephalothorax and abdomen was taken prior to exposure. Six infected shrimp were

assayed. Two groups of 12 unexposed shrimp served as the negative control. Each shrimp from the negative control treatment was fed a piece of uninfected tissue from the cephalothorax or the abdomen. Dissections of uninfected shrimp were performed as above. Viral diagnosis was as in Expts 1 and 2. Only mortality rates were reported for this experiment.

**RESULTS**

**Expt 1. Infectivity from cephalothorax, abdomen, or whole shrimp**

Mean weights of infected cephalothorax, abdomen, and whole shrimp used for exposures, and the mean weights of the infected cephalothorax, abdomen, and whole shrimp remaining at the end of the 24 h exposure period are listed in Table 1. Shrimp that were exposed to an infected abdomen did not consume the entire abdomen. The mortality for shrimp that fed on an infected cephalothorax was estimated to be 0.75, on an infected abdomen 0.40, and on an infected whole shrimp 0.67 (Fig. 1). A significant difference in mortality was detected among shrimp exposed to the infected cephalothorax, abdomen, or whole shrimp (randomization test,  $p = 0.049$ ). The transmission coefficient ( $\beta$ ) estimates for shrimp exposed to the cephalothorax was 0.95, to the abdomen 0.59, and to the whole shrimp cadaver 0.69 (Fig. 2). Most animals that died died between 24 and 60 h post-exposure. All animals that died during the isolation phase and were examined were histologically positive for WSSV. Furthermore, no survivor was histologically positive for WSSV. No animals from the negative control died or were diagnosed with WSSV during this experiment.

**Expt 2. Infectivity from cephalothorax, abdomen, or whole shrimp**

In Expt 1, shrimp that fed on an infected abdomen did not ingest the entire abdomen; therefore in Expt 2,

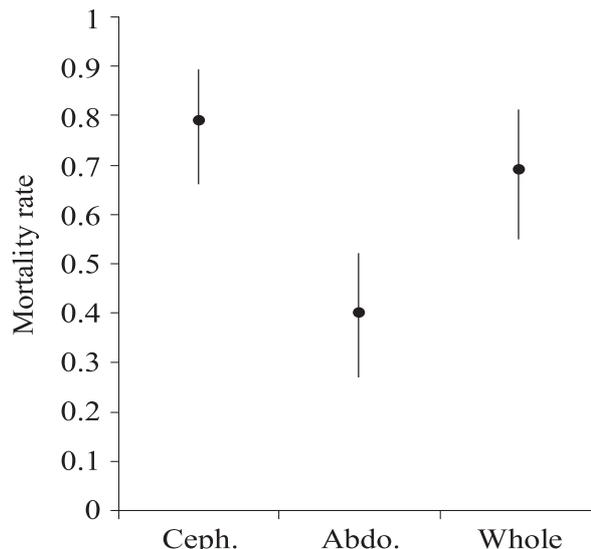


Fig. 1. Mortality rate estimates for *Litopenaeus vannamei* exposed to a WSSV-infected cephalothorax (Ceph.), abdomen (Abdo.), or whole shrimp cadaver from Expt 1. Error bars are the 95% confidence limits estimated by resampling simulation

susceptible shrimp were starved for 3 d. Shrimp consumed the entire infected material in all tanks except one. That tank was excluded from the analysis. Mean weights of infected cephalothorax, abdomen, and whole shrimp used for exposures are listed in Table 1.

The mortality for susceptible shrimp exposed to an infected cephalothorax was 0.58, to an infected abdomen was 0.63, and to an infected whole shrimp cadaver was 0.60 (Fig. 3). Unlike Expt 1, no significant difference in mortality was detected among susceptible shrimp exposed to the infected cephalothorax, abdomen, or whole shrimp (randomization test,  $p = 0.98$ ). The transmission coefficient estimate from an infected cephalothorax was 0.84, from an infected abdomen was 0.83, and from an infected whole shrimp was 0.60 (Fig. 4). Similar to Expt 1, most mortalities occurred between 24 and 60 h post-exposure. Shrimp from 1 tank from each of the cephalothorax, abdomen, and whole shrimp treatments and the 12 negative control ani-

Table 1. Mean weights (g) of WSSV-infected whole shrimp, infected cephalothorax, or infected abdomen fed to susceptible shrimp from Expts 1, 2, and 3, and the mean weight (g) of the infected cephalothorax, abdomen, and whole shrimp remaining at the end of the 24 h exposure period from Expts 1, 2, and 3

Expt	Whole Mean (SD)	Remaining Mean (SD)	Cephalothorax Mean (SD)	Remaining Mean (SD)	Abdomen Mean (SD)	Remaining Mean (SD)
1	1.9 (0.2)	0.5 (0.6)	0.9 (0.1)	0.1 (0.1)	1.0 (0.1)	0.7 (0.3)
2	1.1 (0.1)	0 <sup>a</sup>	0.5 (0.1)	0	0.4 (0.1)	0
3			1.02 (0.3)		0.95 (0.3)	

<sup>a</sup>One replicate excluded because of incomplete ingestion of dose

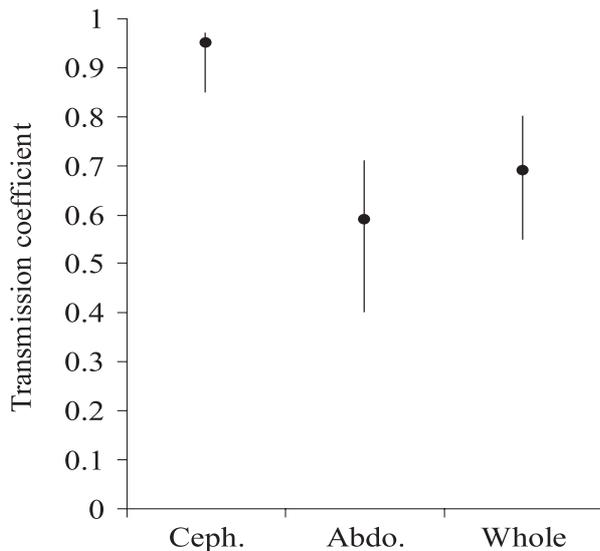


Fig. 2. Transmission coefficient estimates for *Litopenaeus vannamei* exposed to a WSSV-infected cephalothorax (Ceph.), abdomen (Abdo.), or whole shrimp cadaver from Expt 1. Error bars are the 95% confidence limits estimated by resampling simulation

imals were examined histologically for the presence of WSSV intranuclear inclusions. As in Expt 1, all shrimp that died during the isolation phase and were examined were histologically positive for WSSV, and no survivor was histologically positive for WSSV. No animals from the negative control died or were diagnosed with WSSV during this experiment.

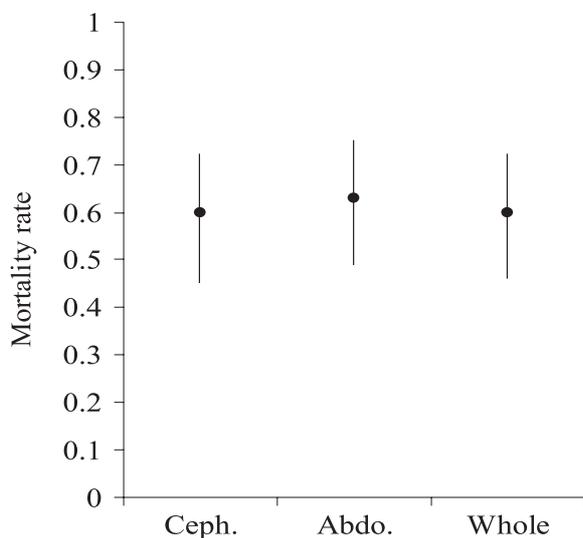


Fig. 3. Mortality rate estimates for *Litopenaeus vannamei* exposed to a WSSV-infected cephalothorax (Ceph.), abdomen (Abdo.), or whole shrimp cadaver from Expt 2. Shrimp were starved for 3 d prior to exposures to ensure complete ingestion of dose. Error bars are the 95% confidence limits estimated by resampling simulations

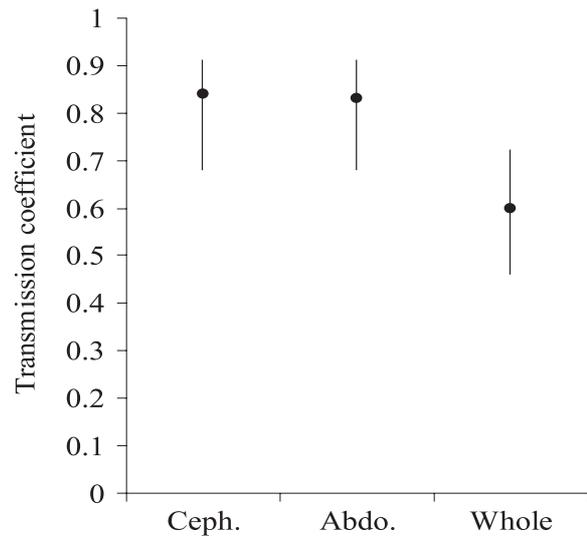


Fig. 4. Transmission coefficient estimates for *Litopenaeus vannamei* exposed to a WSSV-infected cephalothorax (Ceph.), abdomen (Abdo.), or whole shrimp cadaver from Expt 2. Shrimp were starved for 3 d prior to exposures to ensure complete ingestion of dose. Error bars are the 95% confidence limits estimated by resampling simulations

### Expt 3. Infectivity from pieces of cephalothorax or abdomen

Mean weights of infected cephalothorax and abdomen used for exposures are listed in Table 1. The mortality for susceptible shrimp that fed on an infected cephalothorax was estimated to be 0.57 and on an infected abdomen was 0.58 (Fig. 5). No significant difference in mortality estimates was detected between iso-

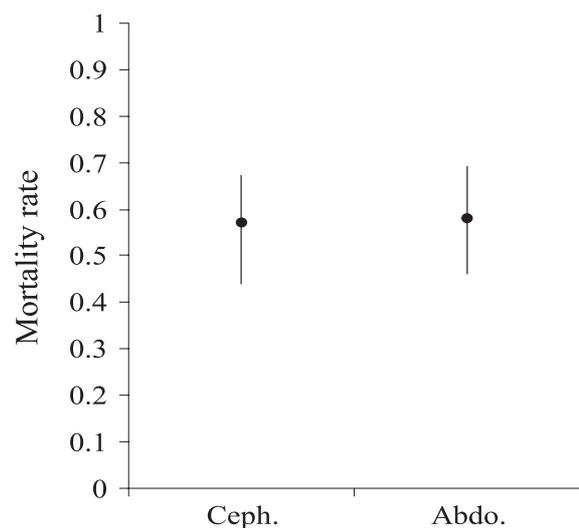


Fig. 5. Mortality rate estimates for isolated *Litopenaeus vannamei* exposed to pieces of WSSV-infected cephalothorax (Ceph.) or abdomen (Abdo.) from Expt 3. Error bars are the 95% confidence limits estimated by resampling simulation

lated susceptible shrimp exposed to pieces of infected cephalothorax or abdomen (randomization test,  $p = 0.89$ ). Like Expts 1 and 2, most mortalities occurred between 24 and 60 h post-exposure. Shrimp from 1 replicate from each of the cephalothorax and abdomen exposures and the 2 negative control groups were examined histologically for the presence of WSSV intranuclear inclusions. As in Expts 1 and 2, all shrimp that died during the isolation phase and were examined were histologically positive for WSSV. Further, no survivor was histologically positive for WSSV. Moreover, no animals from either negative control groups died or were diagnosed with WSSV during this experiment.

## DISCUSSION

Our study compared the relative infectivity resulting from a WSSV-infected cephalothorax to that resulting from a WSSV-infected abdomen. No difference in mortality was detected when susceptible shrimp consumed the same quantity of infected cephalothorax or abdomen (cf. Expts 2 and 3). Similar results were obtained by Tsai et al. (1999), who found by PCR that the viral load of the infected cephalothorax and abdomen was similar.

An assumption implicit in Eq. (1) is random mixing of the susceptible and infected individuals in the exposure tanks (Dwyer 1991, D'Amico et al. 1996, Knell et al. 1996, 1998). In other words, each of the 12 susceptible shrimp must have an equal chance of contacting the infected shrimp. In Expt 2, no difference was observed in the estimated mortality from an infected cephalothorax, abdomen, or whole shrimp cadaver even though the susceptible shrimp that ingested the whole shrimp consumed about twice as much infected tissue (Table 1). Moreover, the estimated transmission coefficient was lower for susceptible shrimp exposed to an infected whole shrimp than to an infected cephalothorax. One possible explanation is that some susceptible shrimp are less aggressive feeders and consume less tissue during the exposure phase than others. Some evidence to support this contention comes from Expt 3, where an infected whole shrimp was dissected into 24 pieces and each piece was fed to 24 individually isolated shrimp. More than 12 infections resulted, indicating that there was enough infected tissue in a whole shrimp to infect 12 shrimp in an exposure tank. The fact that fewer infections occurred in Expts 1 and 2 suggests that some susceptible shrimp may be less aggressive feeders, and so the susceptible shrimp in the exposure tanks are differentially contacting the infected shrimp. Therefore, the assumption of random mixing is violated.

We are not implying that increases in dose ( $I_0$ ) do not yield more infections, only that we did not detect a dif-

ference in the number of infections when susceptible shrimp were exposed to a half or a whole shrimp.

Another assumption of Eq. (1) is that each contact between susceptible and infected individuals is as likely to pass infection as another. For this to be true, each susceptible shrimp must be equally susceptible to the pathogen, and each infected shrimp must be as infectious as another. For pathogens that are transmitted primarily by ingesting portions of infected cadavers, virus must be homogeneously distributed throughout the body and each portion must be equally palatable.

In Expt 1, susceptible shrimp did not completely consume the infected abdomen despite having the same exposure period as the susceptible shrimp feeding on an infected cephalothorax or whole shrimp (Table 1). One reason that the abdomen was less infectious may be because the abdomen is composed primarily of muscle and more difficult for shrimp to tear apart and consume. Therefore, the abdomen was less infectious because it is less palatable. Regardless of the reason, the results indicated that every part of an infected shrimp was not as infectious as another.

In Expt 3, whole infected shrimp were dissected into 24 pieces and fed to 24 isolated susceptible shrimp. Twenty-four infections should have arisen if: (1) each shrimp was equally susceptible, (2) virus was homogeneously distributed throughout the body, and (3) if each portion of the infected shrimp was equally palatable. Less than 24 infections were observed, suggesting that at least 1 of the above was not true. In this experiment the difference in palatability between the cephalothorax and the abdomen was not a factor because each individual shrimp received a piece of infected tissue, and all shrimp ingested the piece of infected tissue by 24 h post-exposure. The susceptible shrimp (*Litopenaeus vannamei*) used in these experiments are from the original unselected population of shrimp (Kona stock) that have been maintained by the United States Marine Shrimp Farming Program at the Oceanic Institute in Hawaii (Lotz 1997). These shrimp have been bred for at least 8 generations and are likely very similar genetically. Consequently, a genetic difference in susceptibility among shrimp was probably not likely. It is more likely that 24 infections did not arise because virus was not homogeneously distributed throughout the cephalothorax and abdomen.

Despite providing some evidence that was inconsistent with some of the assumptions of pathogen transmission used in epidemiology models, the estimation of  $\beta$  is useful because the  $\beta$ s can be used for relative estimates rather than as absolute estimates. Nonetheless, determining how pathogen transmission for WSSV and *Litopenaeus vannamei* deviates from those assumptions is of importance to the development of models of WSSV epidemics.

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