

NOTE

Differences in the susceptibility of some penaeid prawn species to gill-associated virus (GAV) infection

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ABSTRACT: Four species of penaeid prawn cultured in Australia (*Penaeus monodon*, *Penaeus esculentus*, *Marsupenaeus japonicus* and *Fenneropenaeus merguensis*) were injected with a virulent preparation of gill-associated virus (GAV). *P. monodon* (average weight = 8.9, 13.9 and 19.2 g), *P. esculentus* (average weight = 19.5 g), *F. merguensis* (average weight = 10.5 g), and small (average weight = 5.8 g) *M. japonicus* displayed overt signs of disease and mortalities which reached 82 to 100% within 23 d post-injection. Cumulative mortalities in *P. esculentus* and *F. merguensis* were significantly lower than for *P. monodon* of the same size class. Medium (average weight = 13.0 g) *M. japonicus* also developed overt signs of disease but cumulative mortalities were not significantly higher than uninfected controls. Large (average weight = 20.3 g) *M. japonicus* did not display symptoms of disease and there were no significant mortalities up to 23 d post-injection.

KEY WORDS: Gill-associated virus, GAV · Penaeid prawn · Disease

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Gill-associated virus (GAV) has caused stock losses to the *Penaeus monodon* culture industry in Australia at least since 1996. Diseased *P. monodon* infected with GAV display pink to red colouration of the body and appendages, and pink to yellow colouration of the gills. Other signs of disease include lethargy, lack of appetite, secondary fouling and tail rot (Spann et al. 1997). Morphologically, GAV resembles yellow-head virus (YHV) from Thailand (Boonyaratpalin et al. 1993). GAV and YHV virions are rod-shaped, enveloped particles containing helical nucleocapsids that mature by budding at intracytoplasmic membranes (Chantanachookin et al. 1993, Spann et al. 1997). Nucleotide sequence comparisons of the putative polymerase (ORF1b) genes have indicated that GAV and

YHV are closely related but distinct viruses and are likely to be classified in the order *Nidovirales*, possibly in the family *Coronaviridae* (Cowley et al. 1999, 2000a).

Lymphoid organ virus (LOV) has also been described as a rod-shaped, enveloped RNA virus that is endemic in healthy wild and cultured *Penaeus monodon* in Queensland (Spann et al. 1995). Screening of broodstock collected in northern Queensland has indicated a prevalence of LOV infection that exceeds 96% (Cowley et al. 2000b). LOV-infected prawns show no visible symptoms of disease, but lymphoid organs typically contain discrete foci (spheroids) of hypertrophied, infected cells. Spheroids are not typically associated with GAV infection in which there is extensive necrosis of lymphoid organ tissue (Spann et al. 1997). Nucleotide sequence comparison of regions in the putative polymerase genes of multiple GAV and LOV isolates has indicated that they are genetically indistinguishable populations (Cowley et al. 2000b). GAV and LOV can be regarded as the same virus, which causes either overt or covert infections in *P. monodon*. In this paper the name GAV will be used for both overt and covert states of the infection.

*Marsupenaeus*¹ *japonicus* (also called *Penaeus japonicus*) is the second most common penaeid species cultured in Australia. Although cultured in areas where *P. monodon* is also farmed, there are no reports of overt or covert GAV infection in *M. japonicus*. *Penaeus esculentus* and *Fenneropenaeus merguensis* (also called *Penaeus merguensis*) have also been cultured in Australia without evidence of GAV infection. Indeed, screening of wild and cultured penaeids using the sensitive RT-nested PCR test (Cowley et al. 2000b) has indicated that *P. monodon* is the only known natural host of GAV in Queensland. In this paper, we

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¹Genus and species names used in this paper are according to the taxonomic revision of Pérez-Fanfante & Kensley (1997)

examine the susceptibility of these 4 species of penaeid prawn to experimental GAV infection and disease.

Materials and methods. Preparation of inoculation and experimental infections: A standard extract of GAV-infected prawn tissue was used as an inoculum in all experiments. The inoculum was prepared as described previously (Spann et al. 1997) from prawns collected during an outbreak of disease on a farm in northern Queensland in 1996. A total of 25 sub-adult *Penaeus monodon* were infected with a filtered extract of GAV. At 5 d post-injection (p.i.), 11 of the 25 prawns remained alive and displayed symptoms associated with overt GAV infection. Two of these prawns were fixed for examination by transmission electron microscopy (TEM) to confirm the presence of GAV. The 9 remaining prawns were used for the preparation of the inoculum.

The cephalothoraces of 9 GAV-infected prawns were immersed in 6 volumes of lobster haemolymph medium (LHM; Paterson & Stewart 1974) following removal of the carapace and calcareous mouth parts. A total of 55.6 g prawn tissue in LHM was homogenized on ice using an Ultra-turrax tissue grinder (Janke & Kunkel, Ika-werx, Staufen, Germany). The homogenate was clarified at $1300 \times g$ for 5 min at 4°C and the supernatant further clarified at $18000 \times g$ for 20 min at 4°C. Supernatant below the lipid layer was divided into 1.5 ml aliquots, snap frozen and stored at -70°C. For all experimental infections, aliquots of the inoculum were thawed rapidly in a 37°C water bath, passed through a 0.2 µm filter and maintained on ice prior to inoculation. Each experimental prawn was injected with 5 µl g⁻¹ body weight into the second abdominal segment using a 26-gauge needle.

Source of experimental prawns and experimental conditions: Healthy *Penaeus monodon*, *P. esculentus* and *Marsupenaeus japonicus* were collected from 2 farms in south-eastern Queensland and 1 farm in northern Queensland. Healthy *Fenneropenaeus mer-*

guiensis were captured from the Logan River in south-eastern Queensland. On arrival, 3 prawns of each species from each location were dissected and the lymphoid organs examined by light microscopy for existing GAV infection. Prawns were maintained in 100 l circular plastic tanks of sea water at a salinity of 27 ppt and a temperature of 26°C. They were stocked at a density of 8 prawns tank⁻¹ in 60 l of water. Water was partially exchanged and the prawns were fed pelleted food daily.

Host susceptibility to GAV: The susceptibility to GAV of prawns from different penaeid species and different size ranges was tested. The species, size ranges and average sizes are shown in Table 1. Of 32 prawns used in each experiment, 16 were injected with GAV and 16 were injected with LHM. The prawns were monitored daily for overt signs of disease and mortalities recorded.

Results and discussion. GAV-free populations of *Penaeus monodon* have not yet been identified in Australia. The healthy *P. monodon* used in this study displayed histological characteristics typical of covert GAV (i.e. LOV) infection. Lymphoid organs displayed normal tubule structure and contained spheroids of hypertrophic, infected cells (Spann et al. 1995). By light microscopy, there was no evidence of spheroid formation in *P. esculentus*, *Marsupenaeus japonicus* or *Fenneropenaeus merguensis* used in these experiments. Screening of wild and farmed stocks by RT-PCR has also failed to detect evidence GAV infection in any penaeid species other than *P. monodon* (J.A.C. and colleagues unpubl. data).

Covertly infected *Penaeus monodon* of different size classes (Table 1) were tested for susceptibility to disease following super-infection with the virulent GAV standard inoculum. Following experimental infection, *P. monodon* from all 3 size classes displayed typical gross signs of GAV infection including red colouration of the appendages, tail fan and mouth parts, and yel-

Table 1. Species and size classes of penaeid prawns investigated for susceptibility to disease following intramuscular injection with GAV

Species	Size class	Range (g)	Average (g)	Number of prawns
<i>Penaeus monodon</i>	Small	6.0–10.8	8.9	32
	Medium	11.2–16.1	13.9	32
	Large	17.7–20.1	19.2	32
<i>Marsupenaeus japonicus</i>	Small	3.8–7.2	5.8	32
	Medium	12.0–14.1	13.0	32
	Large	18.1–22.8	20.3	32
<i>Penaeus esculentus</i>	Large	15.5–22.5	19.5	32
<i>Fenneropenaeus merguensis</i>	Small-medium	4.2–17.8	10.5	32

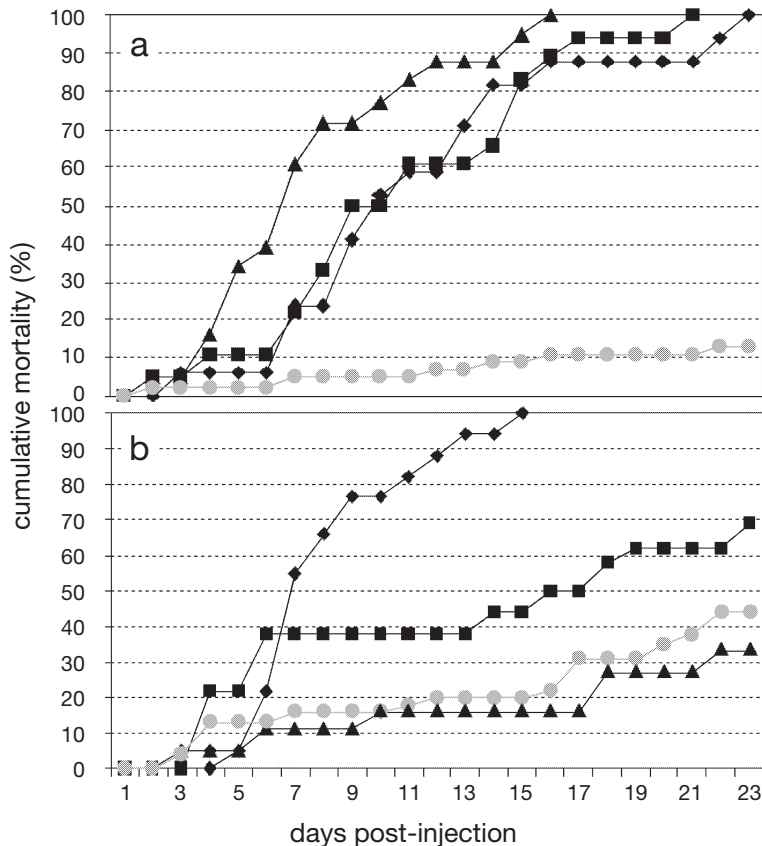


Fig. 1. Cumulative mortality of small (◆), medium (■) and large (▲) *Penaeus monodon* (a) and *Marsupenaeus japonicus* (b) following injection with a virulent preparation of GAV. Cumulative mortality for negative control prawns from the 3 size classes injected with lobster haemolymph medium (LHM) were pooled (●) for each species

low to pink colouration of the gills (Spann et al. 1997). These symptoms were evident in some prawns from Day 6 p.i. and in all prawns by Day 12 p.i. Lethargy, lack of appetite and tail rot were also commonly observed. Cumulative mortalities for the 3 size classes of *P. monodon* are shown in Fig. 1a. Cumulative mortalities reached 100% within 16 to 23 d p.i. for all 3 groups and no size related resistance to disease was observed.

Healthy *Marsupenaeus japonicus* of different size classes (Table 1) were also tested for susceptibility to disease following experimental infection with the virulent GAV standard inoculum. Following infection, small (average weight = 5.8 g) and medium (average weight = 13.0 g) *M. japonicus* displayed abnormal orange body colouration from Day 9 p.i., but the gills remained normal in appearance.

Small *M. japonicus* also displayed lethargy and a lack of appetite. Large (average weight = 20.3 g) experimentally infected *M. japonicus* displayed no gross signs of disease. Significantly different cumulative mortalities were observed between small *M. japonicus* and LHM-injected controls (Fig. 1b; logistic regression coefficient = -4.5560 ; $p > 0.003$, chi-squared test). However, cumulative mortalities for medium and large *M. japonicus* were not significantly different from uninfected controls (logistic regression coefficients were -0.1133 [$p > 0.925$, chi-squared test] and -0.4700 [$p > 0.632$; chi-squared test] respectively).

Large healthy *Penaeus esculentus* (average weight = 19.5 g) were also tested for susceptibility to disease following experimental infection with the virulent GAV standard inoculum. Approximately 40% of infected *P. esculentus* displayed varying degrees of pink to red body, gill and appendage colouration from Day 6 p.i. Lethargy and a lack of appetite were also observed. Cumulative mortalities reached 82% by Day 10 p.i. (Fig. 2) and occurred at a similar rate to *P. monodon* until Day 14 p.i. However, unlike *P. monodon*, some *P. esculentus* had survived this dose of GAV at the termination of the experiment on Day 23. The histology of

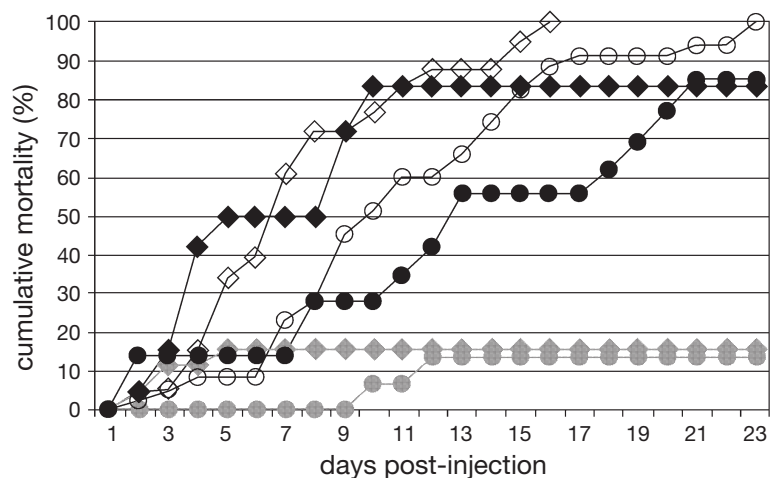


Fig. 2. Cumulative mortality of *Penaeus monodon*, *P. esculentus* and *Fenneropenaeus merguensis* injected with either a virulent preparation of GAV or LHM. (◆, ◇) *P. esculentus* injected with GAV and LHM, respectively. (●, ●) *F. merguensis* injected with GAV and LHM, respectively. (○) Large *P. monodon* injected with GAV. (○) Combined small and medium *P. monodon* injected with GAV

GAV infection in these survivors and their susceptibility to super-infection with GAV is the subject of ongoing study. When estimated by non-linear regression, the difference between the slopes of mortality curves (t -value = 2.40, $p < 0.01$) for *P. esculentus* and *P. monodon* was marginally significant and the difference between the turning points of the mortality curves (t -value = 0.12) was not significantly different. This shows that mortalities for these species commenced at the same time, but that significantly fewer *P. esculentus* died. Therefore, *P. esculentus* may be slightly more resistant to the development of overt GAV infection than *P. monodon*. However, both of these species were far more susceptible to disease than large *Marsupenaeus japonicus* (Fig. 1b).

Healthy, small to medium *Fenneropenaeus merguensis* (average weight = 10.5 g) were also investigated for susceptibility to GAV infection. Following experimental GAV infection, *F. merguensis* displayed pink colouration of appendages and body surface, lethargy and a lack of appetite by Day 9 p.i. Cumulative mortalities reached 86% by Day 21 p.i. and remained lower than for *Penaeus monodon* of the same size (Fig. 2). When estimated by non-linear regression, the slopes (t -value = 8.18, $p < 0.001$) and turning points (t -value = 4.80, $p < 0.001$) of the mortality curves for *F. merguensis* and *P. monodon* were significantly different. This indicates that cumulative mortalities for *F. merguensis* occurred at a slower rate and later in time than for *P. monodon* of the same size. *F. merguensis* appears to be more resistant to the development of overt GAV infection than *P. monodon*. Due to the unavailability of larger animals, the relationship between size and susceptibility to disease was not determined for *F. merguensis*.

In this study, we show that *Penaeus monodon*, *P. esculentus*, *Marsupenaeus japonicus*, and *Fenneropenaeus merguensis* are susceptible to GAV infection and develop disease. It has been established previously that GAV from Australia and YHV from Thailand are distinct but closely related viruses (Spann et al. 1997, Cowley et al. 1999). *P. monodon* is a natural host of both GAV in Australia and YHV in Thailand. Although GAV has not been reported in stocks of *M. japonicus* cultured in Australia, a yellow head-like virus was identified in 1 to 3 g *M. japonicus* from Taiwan which were co-infected with white spot syndrome virus (WSSV; Wang et al. 1996). *P. monodon* stocks from Taiwan have also been shown to be co-infected with YHV and WSSV (Wang & Chang 2000). *F. merguensis* from Thailand has been infected experimentally with YHV, and has been implicated as a carrier of latent YHV infection in prawn culture ponds (Flegel et al. 1996). The susceptibility of *P. esculentus* to infection by YHV is not known. Four penaeid species

cultured in the Americas, *Farfantepenaeus aztecus*, *Farfantepenaeus duorarum* (also called *Penaeus aztecus* and *P. duorarum*), *Litopenaeus vannamei* and *Litopenaeus stylirostris* (also called *Penaeus vannamei* and *P. stylirostris*) are not known to be natural hosts of YHV, but are susceptible when injected with or fed YHV-infected tissue (Lu et al. 1994, Lightner et al. 1998). *Metapenaeus ensis*, *Palaemon styliiferus* and *Euphausia superba* have also been reported to be natural hosts of YHV in Thailand (Flegel 1997).

The data presented in this paper also indicate that there are differences in the susceptibility of penaeid species to disease following GAV infection. *Penaeus monodon*, the natural host, was most susceptible to disease and associated mortality. *P. esculentus* and *Fenneropenaeus merguensis* were marginally less susceptible than *P. monodon*. In *Marsupenaeus japonicus*, there was a size-related resistance in which prawns smaller than 12 g were more likely to develop disease. It is possible that disease may have been delayed in larger *M. japonicus*. However, there was no significant mortality in 18 to 23 g prawns at 23 d post-injection, at which time the cumulative mortalities in small *M. japonicus* and all size classes of *P. monodon* had reached 100%. Although the mechanism of size-related resistance to GAV infection in *M. japonicus* is unknown, subsequent RT-nPCR studies have shown that surviving prawns are infected and appear to remain infected but healthy for several months after inoculation (K.M.S. and colleagues unpubl. data). It appears, therefore, that the mechanism relates to susceptibility to disease rather than to infection per se.

Variations in the susceptibility of penaeid species to viral infection have been described previously. Bell & Lightner (1984) have shown that infectious hypodermal and haematopoietic necrosis virus (IHHNV) is more pathogenic in *Litopenaeus stylirostris* than in *Litopenaeus vannamei*. Bell & Lightner (1987) also reported less rapid development of histopathological lesions in larger *L. stylirostris* infected with IHHNV. Taura syndrome virus (TSV) has been reported to be highly pathogenic for *L. vannamei* and *Fenneropenaeus chinensis* (also called *Penaeus chinensis*), less pathogenic for *L. stylirostris* and *Litopenaeus setiferus* (also called *Penaeus setiferus*) and infectious, but non-pathogenic, for *F. duorarum* and *F. aztecus* (Brock et al. 1995, Overstreet et al. 1997). Lotz (1997) found no correlation between size and susceptibility of *L. vannamei* to TSV infection.

All 4 penaeid species examined in this study have been farmed commercially in Australia. *Penaeus esculentus* and *Fenneropenaeus merguensis* have been farmed on a relatively small scale to date. However, as they are not natural hosts of GAV, they have been viewed as possible alternative culture species to *P.*

monodon. The data reported here demonstrate that both species are susceptible to infection by GAV when injected intramuscularly. Other means of infection need to be investigated. However, the results indicate that there is a risk of transmission of GAV when *P. monodon* is cultured in the same vicinity or in polyculture with other penaeid prawns. In Australia, *P. monodon* and other species are sometimes cultured on the same site and it is common for *P. monodon* and *Marsupenaeus japonicus* farms to operate in close proximity and share the same water systems. Care should be taken to avoid cross-contamination with water or moribund animals potentially infected with GAV.

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