Transmission of *Panulirus argus* virus 1 (PaV1) and its effect on the survival of juvenile Caribbean spiny lobster

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ABSTRACT: The Caribbean spiny lobster *Panulirus argus*, an important fisheries species, is host to *Panulirus argus* virus 1 (PaV1), a lethal, unclassified virus—the first found in any species of lobster—prevalent in juvenile lobsters. We describe a series of laboratory experiments aimed at assessing the likely modes of disease transmission, determining the survival of lobsters relative to each transmission pathway and identifying potential alternate hosts. Given evidence for lower prevalence of PaV1 in large lobsters, the effect of lobster size on susceptibility was also examined. Results demonstrated that PaV1 can be transmitted to juvenile lobsters via inoculation, ingestion of diseased tissue, contact with diseased lobsters and—among the smallest juveniles—through water over distances of a few meters. Contact and waterborne transmission, the most likely modes of transmission in the wild, were less efficient than inoculation or ingestion. Nevertheless, about half of the smallest lobsters in contact and waterborne trials contracted the disease and died within 3 mo. Other decapods that co-occur with *P. argus* (e.g. spotted lobster *P. guttatus*, stone crab *Menippe mercenaria*, channel crab *Mithrax spinosissimus*) did not acquire the disease after inoculation with PaV1-infected hemolymph. Our results confirmed that PaV1 is highly infectious and lethal to juvenile *P. argus*, particularly early benthic juveniles in the wild, and, hence, is a threat to mariculture.

KEY WORDS: PaV1 · Disease · Ecology · Mortality · Epidemiology · Outbreak · Infection

INTRODUCTION

Panulirus argus virus 1 (PaV1), the first naturally occurring, pathogenic virus known to infect any species of lobster, infects juvenile Caribbean spiny lobsters *P. argus* throughout the Florida Keys, Florida, USA (Shields & Behringer 2004). PaV1 infections have also been found in juvenile *P. argus* from Belize, Mexico, and the US Virgin Islands; thus, we suspect that the disease is widespread in the Caribbean Sea. The Caribbean spiny lobster occurs throughout the Caribbean Sea and along the Atlantic coast of Central America and South America, from Brazil to Florida and Bermuda (Holthuis 1991). Wherever *P. argus* occurs in abundance, it is heavily exploited by commercial and recreational fishers, supporting valuable fisheries in

Florida and throughout the Caribbean (Hunt 2000, FAO 2001, 2004). Given its pathogenicity, PaV1 represents a serious threat to these important fisheries, which have experienced recent Caribbean-wide declines in catches (FAO 2001, 2004). In addition, outbreaks of PaV1 have resulted in mortalities of juvenile lobsters reared in experimental mariculture facilities in Florida, the Bahamas and Belize, underscoring the potentially serious nature of the pathogen.

Apart from PaV1, other naturally occurring viral infections are unknown in lobsters (Evans et al. 2000, Shields et al. 2006). White spot syndrome virus (WSSV) is pathogenic to several species of spiny lobsters in the laboratory, but it does not occur naturally in these hosts (Chang et al. 1998, Wang et al. 1998, Supamattaya et al. 1998, Rajendran et al. 1999, Syed Musthaq et al.

2006). Pathogenic viruses are, however, relatively common in other decapod crustaceans, such as penaeid shrimps (Lightner & Redman 1998) and blue crabs (Shields & Overstreet 2007). Although viruses infect many crustaceans, few besides the shrimp viruses are well studied.

Infections of 2 pathogens have been examined in spiny lobsters in the laboratory, although neither occurs naturally in spiny lobsters. *Aerococcus viridans*, the causative agent of gaffkemia in clawed lobsters *Homarus* spp. is transmitted through breaks in the cuticle and contaminated muds can serve as fomites for transmission (Stewart et al. 1969, Stewart 1984). When injected, *A. viridans* is pathogenic to the California spiny lobster *Panulirus interruptus* (Schapiro et al. 1974) and to *P. argus* (Bobes et al. 1988), but other modes of transmission have not been examined. The infectivity of the WSSV that infects shrimp has also been experimentally tested in the spiny lobsters *P. homarus, P. longipes*, *P. ornatus*, *P. penicillatus*, *P. polyphagus* and *P. versicolor* via inoculation (Rajendran et al. 1999, Musthaq et al. 2006) and ingestion (Chang et al. 1998, Musthaq et al. 2006). WSSV was detectable in lobster tissues by PCR after inoculation, but not necessarily after ingestion, and mortality attributable to WSSV was inconsistent. However, these studies were marred by low sample sizes, lack of proper controls and insufficient statistical analysis, leaving unresolved the question of pathogenicity of WSSV in spiny lobsters. There is little doubt, however, that the naturally occurring PaV1 virus is pathogenic in *P. argus*.

PaV1 is a large, unenveloped, icosahedral DNA virus with a nucleocapsid approximately 187 nm in size (Shields & Behringer 2004). The virus shares morphological characteristics with the *Herpesviridae* and the *Iridoviridae*, but it is currently unclassified. The virus infects hemocytes (hyalinocytes and semigranulocytes, but not granulocytes), spongy connective tissue cells, some hematopoietic tissues and fixed phagocytes of the host. In the late stages of disease, the normally bluish- or amber-tinted transparent hemolymph of lobsters becomes chalky white with cellular debris. Heavily infected lobsters become moribund and cease normal behaviors, such as grooming, and die from metabolic wasting, typically within 90 d following infection (Shields & Behringer 2004).

Field and laboratory observations suggest that the prevalence of detectable PaV1 infections declines with increasing lobster size (Behringer 2003). Following settlement from the plankton and metamorphosis from the puerulus postlarval stage, the early benthic juvenile (EBJ) dwells solitarily in dense vegetation for several months. The EBJ appears to be highly susceptible to infection by PaV1. The virus spreads rapidly among EBJs housed together in the laboratory, and prelimi-

nary field experiments indicate that the prevalence of PaV1 in wild EBJs may exceed 50% in focal outbreaks (authors' unpubl. data). Upon reaching 15 to 20 mm carapace length (CL; a standard measure of size in lobsters), the juveniles emerge from vegetation, become social and aggregate in crevices provided by sponges, corals and rocks (see Butler et al. 2006). Based on histological evidence, the prevalence of PaV1 is highest (~16%) among the smallest of these crevice-dwelling juveniles (15 to 20 mm CL), declines to about 5% once they reach 35 to 45 mm CL and is nearly imperceptible (<1%) in adults (Shields & Behringer 2004). It is among the normally social crevice-dwelling juvenile lobsters that we have also documented the solitary occurrence of diseased lobsters in the field. Indeed, we demonstrated in laboratory experiments that this occurs because healthy individuals avoid diseased conspecifics even before the virus is transmissible—a previously undocumented behavior in wild animals (Behringer et al. 2006).

Here we describe a series of laboratory experiments designed to determine the most likely pathways by which PaV1 is transmitted among juvenile spiny lobsters. We also tested whether transmission is inversely proportional to juvenile size, as field and laboratory observations suggested. PaV1 was also inoculated into 3 other decapod crustaceans that naturally associate with *Panulirus argus* to determine their susceptibility to infection and disease, and whether they can act as alternate hosts.

MATERIALS AND METHODS

Diagnostic methods. PaV1 infection was diagnosed based on histological examination of prepared tissues. Lobsters were anesthetized in a freezer for 10 to 15 min prior to dissection. Tissues, including hepatopancreas, heart, gills, epidermis (overlying the cardiac region), hindgut, and the dorsal portion of the foregut (for hematopoietic tissues), were sampled, immediately placed in labeled cassettes and fixed in 10% neutral-buffered formalin for 48 to 72 h, after which they were transferred to 70% ethanol until further processing. Tissues were processed through routine paraffin procedures using Harris hematoxylin and eosin Y. The dorsal portion of the foregut was first decalcified in formic acid-sodium citrate for 16 to 20 h prior to histological processing. Lobsters were diagnosed for PaV1 infection on the basis of cellular pathology described previously (Shields & Behringer 2004).

Given our reliance on histological methods for identifying PaV1 infections, the lobsters used in the experiments described below could not be screened for infection prior to inclusion in experiments; therefore,

asymptomatic carriers of the virus may have been included in our experiments, then subsequently expressed disease. However, all of our experiments contained control groups of randomly selected individuals that were not exposed to the treatment of interest. Only treatments with levels of disease statistically greater than the control groups were deemed relevant. The low level of disease or mortality in the control groups (see 'Results') indicated that there were few asymptomatic carriers in the study.

Viral transmission. *Transmission by inoculation*: To confirm the transmissibility of PaV1, 21 juvenile lobsters (31.8 to 54.9 mm CL) were captured from the field, transported to the laboratory, acclimated in aquaria and inoculated with hemolymph from an infected individual. They were maintained in isolation for 80 d in flow-through ambient seawater tanks (44 l capacity) in the laboratory. Healthy individuals (n = 10) were also held under identical conditions to serve as controls. Lobsters were inoculated with either 0.1 ml (n = 10 lobsters) or 0.2 ml (n = 11 lobsters) of raw infected hemolymph using a 1 cc tuberculin syringe fitted with a 27 gauge needle. Viral densities could not be estimated in different doses. Inoculum was injected at the juncture of the basis and ischium of the fifth walking leg after sterilization of the arthrodial membrane with 70% ethanol. Lobsters were then maintained in the laboratory in individual containers and monitored for up to 140 d. During this period, they were fed frozen shrimp and squid ad libitum every 2 d. We terminated the experiment after 80 d, and tissue samples from each surviving lobster were obtained for histological examination. Differences in prevalence between treatments were examined using a 2-way log-linear contingency table analysis.

Transmission by ingestion: To determine whether lobsters contract PaV1 infection by ingestion, 28 lobsters (19.4 to 33.9 mm CL) captured from the field were held in isolated flow-through ambient seawater tanks (44 l capacity) and fed abdominal muscle tissue from infected conspecifics. Lobsters were starved for 10 d prior to the initiation of the experiment to ensure that they would eat infected tissue. Thereafter, once a week for 4 wk, they were fed with approximately 1 g of abdominal muscle tissue from an infected lobster killed just prior to each feeding. Lobsters were fed frozen shrimp and squid every other day at all other times. Eight additional uninfected lobsters were fed a diet of squid and shrimp ad libitum to serve as controls. The experiment was terminated after 80 d, and tissues were obtained from each surviving lobster for histological examination. Differences in disease prevalence between treatments were tested using a 2-way log-linear contingency table analysis.

Contact transmission: Three uninfected juvenile lobsters of different sizes were exposed to a single diseased individual in each of 10 isolated, flow-through ambient seawater tanks (44 l capacity); a total of 40 lobsters (10 diseased and 30 uninfected) were used in the experiment. Three healthy lobsters were held in each of 5 other tanks (15 lobsters total) to serve as controls. Note that control lobsters were visually inspected and presumed to be uninfected, but their status was only determined at the termination of the experiment, when all lobsters were histologically examined. Therefore, some of the lobsters considered uninfected at the start of the study may have been previously exposed to the virus; thus, the need for an unexposed control treatment.

To determine whether smaller lobsters were more susceptible to the virus than larger ones, the 3 healthy lobsters held in each tank with the infected individual were of different size classes (small: 20 to 30 mm; medium: 30 to 40 mm; large: 40 to 50 mm CL), thus comprising a randomized-block design experiment. Lobsters were fed shrimp and squid ad libitum. The experiment was terminated after 80 d, and tissue samples were obtained from each lobster for histological examination.

Infection in the tissues of lobsters was ranked based on the relative number of infected cells in the hepatopancreas (Li et al. 2008) as determined by histological examination of tissue samples: uninfected = 0, lightly infected = 1, moderately infected = 2, heavily infected = 3. To limit potential bias, we further blinded the treatment identities of the lobsters for histological analysis. For the contact transmission experiment, these ranks were then used in a 1-factor randomized-block ANOVA. The factor of interest was lobster size, which had 3 levels: small, medium, and large. The blocks in this analysis were the 9 replicate experimental tanks in which one lobster from each of the 3 size groups was housed with a diseased lobster.

Transmission in water: We conducted 2 laboratory studies to determine (1) whether transmission differed over short distances in seawater, and (2) if the effectiveness of transmission in water varied with lobster size. In the first experiment, we tested whether the distance that water traveled between a tank housing a diseased lobster and tanks holding a single, healthy, large juvenile altered the probability of infection of large juveniles (29.2 to 46.4 mm CL). Seawater flowed from the headtank at 1.2 l min^{-1} into each test tank over one of 3 distances: 0.5, 1.6, or 3.6 m (n = 21 lobsters tested per distance).

In the second experiment, we tested the susceptibility of 2 sizes of lobster (EBJ vs. juvenile) to PaV1 infection when exposed to seawater that traveled 2 m from diseased lobsters serving as a source of infection. Juveniles (22.0 to 36.5 mm CL; n = 21) were housed individually in 8 l tanks and EBJs (5.8 to 15.7 mm CL; n = 43)

were housed individually within 1 l cages placed in the same tanks as the larger juveniles. Seawater flowed into each experimental tank through 1 cm diameter tubing from a 880 l headtank, which contained 2 heavily diseased lobsters. One healthy lobster was also housed in an individual cage in each headtank, 0.1 m from the diseased lobster cages to confirm transmission via contact.

Lobsters were fed shrimp and squid ad libitum every other day. The experiment was terminated after 120 d, and tissue samples were obtained from each lobster for histological examination. Differences in infection and survival among lobster size classes or waterborne distance treatments were analyzed using multi-way log-linear contingency table analyses.

Effect of disease on survival. To quantify the effect of PaV1 infection on juvenile lobster survival, we aggregated data from all comparable laboratory studies to consider the effect of exposure type (i.e. inoculation, contact, ingestion, waterborne), lobster sex, lobster size, and the number of injuries per lobster on survival. A Kaplan-Meier survival analysis was used to compute life tables and to compare survival curves for each type of exposure. Cox regression analysis (both forced-entry and forward-stepwise approaches) was used to examine the independent effect of exposure type, sex, size, and injuries on survival.

Alternate hosts. Three decapod species that commonly co-occur in dens with *Panulirus argus* were used to test the pathogenicity of PaV1 and host range of the virus. Thirty spotted lobsters *P. guttatus* (Decapoda: Palinuridae), 30 stone crabs *Menippe mercenaria* (Decapoda: Xanthidae) and 20 channel clinging crabs *Mithrax spinosissimus* (Decapoda: Majidae) were collected from hard-bottom habitats and coral reefs in the Florida Keys, USA. In the laboratory, we inoculated (0.1 to 0.2 ml) half of the individuals collected for each species with hemolymph from an uninfected lobster and half with hemolymph from a visibly diseased lobster. All were held for 90 d in outdoor tanks (separated by species) supplied with filtered seawater. Every other day, spotted lobsters and stone crabs were fed frozen shrimp and squid, whereas the herbivorous channel crabs were provided with algae. After 90 d, individuals were sacrificed and their tissues prepared for histological examination as described above.

Our various experiments were conducted throughout the year at ambient seawater temperatures (19 to 30°C), so water temperature is an uncontrolled variable in these experiments. We are now performing a more definitive set of experiments to test for temperature effects on lobster susceptibility to PaV1, but field surveys of PaV1 prevalence suggest that it is unrelated to season and presumably temperature (Behringer 2003).

RESULTS

Viral transmission

Infection by PaV1 was achieved in all 4 modes of transmission that were tested. However, inoculation was the most effective mode and waterborne exposure the least effective mode of transmission (Fig. 1).

Transmission by inoculation

Nearly all (95%) of the lobsters injected with hemolymph from PaV1-infected conspecifics became infected and 38% died within 80 d. Infection (goodness-of-fit $G = 20.05$, df $= 1$, p < 0.0005) and mortality ($G = 4.186$, df $= 1$, p $= 0.041$) of PaV1-injected lobsters was significantly greater than that of control lobsters injected with non-infected hemolymph. All of the control lobsters survived, although one showed signs of PaV1 infection at the end of the trial. This single infection among the control lobsters was likely obtained in the field prior to the experiment.

Transmission by ingestion

Significantly more lobsters (42%) became infected with PaV1 after ingestion of PaV1-infected tissue than did control lobsters (0%) that ate uninfected prey ($G = 7.586$, df $= 1$, p $= 0.006$). Evidence that the PaV1 dis-
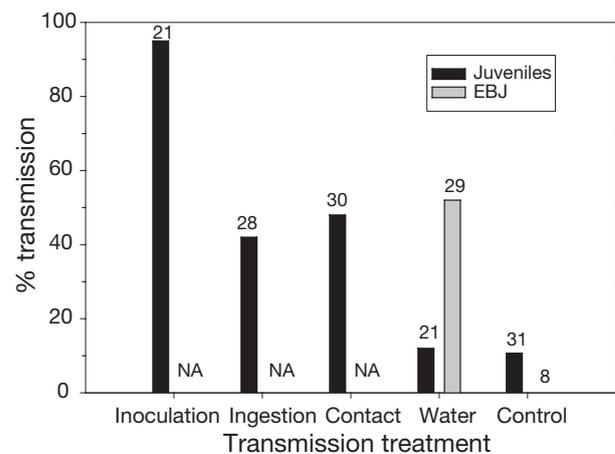

Fig. 1. *Panulirus argus.* Transmission of *P. argus* virus 1 (% of individuals infected 80 d after initial exposure) by 4 different modes of transmission (inoculation, ingestion, contact, waterborne) compared to unexposed controls for juvenile lobsters (25 to 45 mm carapace length, CL) and early benthic juvenile lobsters (EBJ, 5 to 15 mm CL). Transmission in EBJs was not tested by inoculation, ingestion, or contact. Sample sizes given above each histogram

ease is more virulent among the earliest juvenile stages was also seen in this trial, wherein 5 of the 6 smallest lobsters contracted the disease and 2 died during the course of the experiment.

Transmission by contact

Histological examination of the lobsters that were alive at the termination of the contact transmission experiment indicated that many of those exposed to infected conspecifics became infected. However, the effectiveness of contact transmission declined significantly ($F_{2,8}$ = 4.678, p = 0.026) with increasing size; 63% of the small lobsters (20 to 30 mm CL), 33% of the medium lobsters (30 to 40 mm CL) and 11% of large lobsters (40 to 50 mm CL) became infected with PaV1 after 80 d. Two of the 15 control lobsters, both from the small-size group, were diagnosed with PaV1 infections after 80 d. These individuals, obtained from the field, were likely infected prior to the experiment because none of the other controls showed signs of disease.

Waterborne transmission

The prevalence of PaV1 infection among large juveniles (12% infected) in our first waterborne experiment did not differ from background levels of infection (10.7% infected) seen in control animals (G = 0.130, df = 1, p = 0.718). Therefore, PaV1 infection in large juveniles did not differ from controls regardless of the distance that the water traveled (i.e. 0.5, 1.6 or 3.6 m; G = 0.468, df = 2, p = 0.791).

The second waterborne transmission experiment tested the susceptibility of 2 sizes of lobster to PaV1 transmitted 2 m through seawater. The EBJs (<15 mm CL) were significantly more likely to become infected (G = 9.286, df = 1, p < 0.002) or die (G = 4.504, df = 1, p = 0.034) than their larger counterparts (>35 mm CL). About 52% of the EBJs acquired infections versus only 10% of the larger juveniles. Another 33% of the EBJs died before the experiment concluded, whereas only 9.5% of the larger juveniles died. Small and large juvenile lobsters held in head tanks 0.5 m from diseased lobsters experienced similar incidences of infection (i.e. 50% of small juvenile lobsters acquired infection over the time course of the experiment, whereas none of the larger juveniles did) as those exposed in tanks at a distance

of 2 m. The incidence of disease among EBJs (52% infected over the course of the experiment) was significantly higher (G = 9.763, df = 1, p = 0.002) than that for control individuals reared in UV-treated water (n = 8, 0% infected). These results confirmed that waterborne viral transmission occurred over a distance of at least 2 m, but only for EBJs; larger juveniles were much less susceptible to the virus transmitted in seawater.

Calculation of transmission coefficients

The transmission coefficient (β) used in mass-action models (d'Amico et al. 1996, Soto & Lotz 2001, Lotz & Soto 2002) was calculated as:

$$\beta = 1 - \exp\left(\frac{\ln\left(\dfrac{S_i}{S_0}\right)}{I_0}\right) \qquad (1)$$

where I_0 is the initial number of infected lobsters, S_0 is the initial number of susceptible lobsters, and S_i is the number of susceptible (or uninfected) lobsters remaining at time i (the end of the experiment). The unit of measure is the number of new infections per exposure to infected lobsters over the course of the experiment. The variances of the transmission coefficients in the different treatments were calculated using the delta method (Seber 1982). The transmission coefficients varied widely between studies, but the coefficient for contact transmission was greater than those in the waterborne and ingestion modes of transmission (Table 1). Size was again a significant factor for transmission because the smallest lobsters had higher transmission coefficients in both the contact and the waterborne transmission studies, although the confidence intervals (CIs) were large in some treatments. The small EBJs in the waterborne transmission experiment

Table 1. *Panulirus argus*. Transmission coefficients (β) calculated for juvenile lobsters of different sizes when exposed in laboratory experiments to *P. argus* virus 1 via 4 different mechanisms of transmission. Coefficients were calculated from the formula used in mass-action models (d'Amico et al. 1996, Soto & Lotz 2001). CL: carapace length

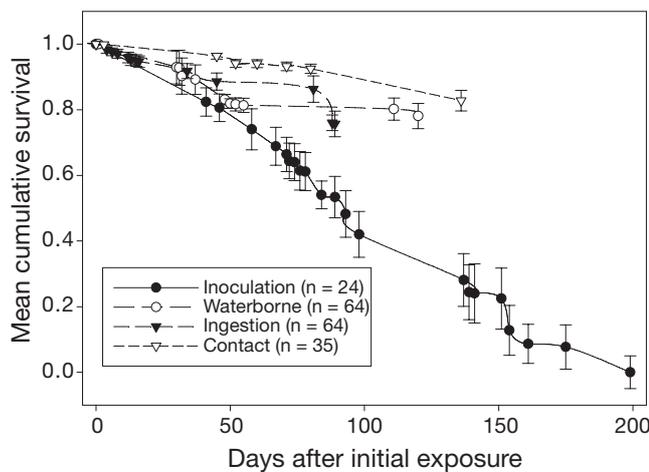| Transmission	Size (CL)	β	95% CI
Inoculation	Small–Large (31.8–54.9 mm)	0.135	0.051–0.219
Ingestion	Small–Large (19.4–33.9 mm)	0.005	0.002–0.008
Contact	Small (20–30 mm)	0.115	0.013–0.218
	Medium (30–40 mm)	0.044	0.000–0.100
	Large (40–50 mm)	0.013	0.000–0.042
Waterborne	Small (22.0–36.5 mm)	0.026	0.012–0.039
	Medium (29.2–46.4 mm)	0.004	0.000–0.009
Contact control	Small (20–30 mm)	0.159	0.123–0.195

Fig. 2. *Panulirus argus.* Cumulative survival curves (means ± SE) for juvenile lobsters (25 to 45 mm carapace length) exposed to P. argus virus 1 infection via 4 different modes of transmission (inoculation, ingestion, contact, waterborne) as estimated by Kaplan-Meier survival analysis. Lobsters serving as controls are not shown because they did not experience mortality

had a significantly higher transmission coefficient than the medium-sized lobsters, and the small lobsters serving as controls for contact transmission had significantly larger transmission coefficients than the other 2 treatments (based on no overlap in CI). The initial number of infected lobsters (I_0) for the inoculation (20) and ingestion (112; 4 feedings for 28 lobsters) trials was based on the number of exposures as opposed to the number of infected individuals. The I_0 for the contact and waterborne transmission experiments were more realistic in that infected individuals served as the source of infections and lobsters were exposed over the course of the experiments.

Effect of disease on survival

All of the uninfected juvenile lobsters (n = 31) that served as controls in our studies survived. Therefore, they were not included in the analyses of survival, thus allowing for a more useful comparison of differences in survival among the 4 modes of disease transmission. A Kaplan-Meier survival analysis comparing the survival of juvenile lobsters (25 to 45 mm CL) exposed to PaV1 via the 4 modes of transmission tested revealed significant differences in lobster survival in relation to mode of transmission

(Tarone-Ware statistic = 8.18, df = 3, p = 0.042). Inoculation of PaV1 into lobsters resulted in the highest mortality, whereas contact and waterborne transmission resulted in significantly lower mortality (Fig. 2). There were no differences in survival between males and females (Tarone-Ware statistic = 0.56, df = 1, p = 0.455).

We also used Cox regression analysis (both forced-entry and forward-stepwise approaches) to examine the independent effect of exposure type, size and injuries on lobster survival. The effect of sex on survival was not considered in the Cox analysis because (1) it had no effect on survival based on the Kaplan-Meier analysis; (2) it contributed very little to the Cox Regression result; and (3) it was not recorded in a number of instances when lobsters were too small and their sex difficult to determine. We evaluated the proportional-hazards model assumption by inspecting plots of the cumulative hazard and log-minus-log functions over time, both of which revealed parallel curves among treatments, indicating that our data did not violate this assumption. Injury had no effect on survival in the Cox regression, but the type of exposure to PaV1 and lobster size significantly altered survival whether we used a forced-entry model χ^2 = 23.411, df = 5, p < 0.0005) or a forward-stepwise model χ^2 = 23.146, df = 4, p < 0.0005) (Table 2).

Alternate hosts

All of the 30 stone crabs and 20 channel crabs that we tested survived the 90 d experiment, as did all but one of the 30 spotted spiny lobsters. None of the crabs or lobsters, including the single lobster that died, presented any sign of PaV1 infection as determined from histological examination of heart, gill, hindgut, foregut, hepatopancreas or connective tissue samples.

Table 2. *Panulirus argus.* Results of a forced-entry Cox Regression evaluating the effect of lobster size (carapace length, CL), number of injuries (injury), and type of disease transmission (inoculation, ingestion, waterborne, contact) on juvenile lobster survival in laboratory experiments. The overall test considering all variables was significant χ^2 = 23.411, df = 5, p < 0.0005). B: slope; Wald: Wald test statistic; Exp(B): slope exponent

	B	SE	Wald	df	p	Exp(B)
CL	−0.039	0.012	11.096	1	0.001	0.962
Injury	0.112	0.208	0.289	1	0.591	1.118
Inoculation			17.882	3	0.000	
Ingestion	2.133	0.644	10.954	1	0.001	8.441
Waterborne	1.581	0.677	5.451	1	0.019	4.859
Contact	0.481	0.658	0.535	1	0.464	1.618

DISCUSSION

PaV1 is infectious and lethal to juvenile lobsters *Panulirus argus*, particularly to EBJs and juveniles up to 25 mm CL. PaV1 can be transmitted among juvenile lobsters via inoculation, through ingestion of diseased tissue, and through close contact with infected individuals. Among the EBJs and juveniles <25 mm CL, the virus can also be transmitted through seawater over distances of at least a few meters. Contact and waterborne transmission—the more likely routes, along with ingestion, of natural transmission—were the least efficient modes of transmission in our experiments. Nonetheless, about a third of the EBJs and small lobsters in the contact and waterborne trials contracted the disease and died within 3 mo. Survival of infected juvenile lobsters was lowest among those inoculated with PaV1 and those that ingested infected tissues. We also confirmed that susceptibility of juvenile lobsters to disease by PaV1 declines with increasing size (age). The virus does not appear to be pathogenic in at least 3 other large decapod crustaceans (i.e. spotted spiny lobster, stone crab, channel crab) that are sympatric with, have similar den requirements to, and are often found cohabiting with *P. argus*. One of these, the spotted spiny lobster *P. guttatus,* is a congener of *P. argus*, indicating a high degree of host specificity by PaV1.

All of the infections in this study were confirmed using histology, which has proven to be an effective diagnostic tool for detecting the characteristic pathological signs of PaV1 infection and disease (Shields & Behringer 2004). Histological examination may not be a sensitive method for detecting the early stages of viral infection, but molecular methods were not available at the time of our experiments. A fluorescence *in situ* hybridization (FISH) assay (Li et al. 2006) and PCR DNA probe (Montgomery-Fullerton et al. 2007) have recently been developed for detection of PaV1 in tissue and hemolymph samples, respectively. Further, a cell culture-based quantal assay has been developed to quantify viral load in hemolymph samples (Li & Shields 2007). None of these techniques had been fully assessed in time for the experiments described here.

Routes of transmission

In nature, the spread of pathogens among marine species occurs in numerous ways, including ingestion of diseased tissue, contact with diseased organisms, and transfer of pathogens through water among others. PaV1 was successfully transmitted by all of these routes in our study, although the efficiency of transmission varied, particularly with host size. Ingestion and

subsequent invasion of pathogens through the intestinal wall is one of the most direct means of disease transmission in crustaceans (Bang 1983, Supamattaya et al. 1998, Wang et al. 1998, Syed Musthaq et al. 2005). We found that PaV1 is effectively transmitted (42%) through ingestion of diseased tissue to juvenile lobsters and that survival in this treatment group was among the lowest of those we tested. In the laboratory, spiny lobsters will resort to cannibalism if held under confined conditions with scarce food (Colinas-Sanchez & Briones-Fourzán 1990), but the degree to which they engage in cannibalism or consume the carcasses of dead conspecifics in nature is unknown. Among the social spiny lobsters it is more likely that if ingested, the viral source is infected prey. Juvenile spiny lobsters are voracious predators of a variety of small benthic prey such as mollusks, echinoderms, crustaceans and polychaetes (Herrnkind et al. 1988, Briones-Fourzán et al. 2003), any of which could potentially serve as a reservoir for the virus, as might benthic sediments or detritus upon which many of these prey feed. We are investigating these as possible sources for PaV1. Alternate hosts can also provide a reservoir of pathogens sometimes necessary to sustain disease in species with spatially segregated populations (e.g. metapopulations; Gog et. al 2002). However, we were unable to transmit PaV1 disease via inoculation to 3 other decapod species that all associate with *Panulirus argus* in the wild, one of which is a congener of *P. argus*, so we view this as an unlikely route of PaV1 transmission.

Transmission of pathogens by contact is likely among social species, but behavior may be an important modulator of disease transmission in the wild. Spiny lobsters, for example, exhibit ontological changes in sociality and react to diseased conspecifics in ways that probably diminish transmission. Shortly after settlement from the plankton, they begin their benthic life as asocial, largely solitary EBJs (Childress & Herrnkind 1994, 1996, reviewed in Butler et al. 2006). The risk of predation for defenseless EBJs is high, but is diminished by their camouflage coloration and solitary existence (Herrnkind & Butler 1986, Butler et al. 1997, 1999, Sharp et al. 2000). EBJs are also highly susceptible to PaV1 infection, which is highly contagious among EBJs. Thus, the asocial nature of EBJs not only reduces their risk of predation in nature, it may also have evolved as a mechanism to reduce their susceptibility to communicable pathogens such as PaV1. Behavior plays a surprisingly different role in regulating PaV1 transmission in *Panulirus argus* among the larger, social juveniles.

Among the normally social large juveniles, healthy lobsters avoid contact with diseased lobsters and do so a week or more before diseased lobsters become

infectious (Behringer et al. 2006). Field observations suggest that such behavior may retard PaV1 transmission in the wild because there is no relationship between local population density and PaV1 prevalence in natural habitats or in habitats augmented with artificial structures that tend to attract and concentrate lobsters (Behringer & Butler 2006). Models of disease dynamics rarely take into account ontogenetic changes in host behavior and patterns of aggregation that potentially affect their susceptibility to disease (McCallum et al. 2001). We have developed such a model for *Panulirus argus* (T. Dolan & M. Butler unpubl. data) to examine how host behavior, habitat structure, and fishery practices influence disease dynamics with hopes that our results are of broad applicability.

The estimation of the transmission coefficients for mass action models similarly requires caution, because transmission is unlikely to be a constant and may be affected by host or pathogen densities, viral dose or other factors that affect host susceptibility (d'Amico et al. 1996, Knell et al. 1998, Behringer et al. 2006). Nonetheless, transmission coefficients provide a starting point for comparisons among possible avenues of transmission or among species or disease systems. For example, when the shrimp *Litopenaeus vannamei* cohabited with infected conspecifics (i.e. contact transmission), the transmission coefficient for WSSV was much lower than what we calculated for contact transmission in PaV1 (see Soto & Lotz 2001). In contrast, the transmission coefficients for WSSV and Taura syndrome virus (TSV) in ingestion trials were higher (0.38 to 0.85) depending upon the species of shrimp (Soto & Lotz 2001, 2003, Lotz & Soto 2002). The transmission coefficients we estimated for ingestion of PaV1 by lobsters were much lower than for WSSV and TSV, which suggests that ingestion is an inefficient route of PaV1 transmission. This is perhaps due to a low degree of cannibalism in spiny lobsters, or it could also be due to differences in sample size, which influence the coefficient.

For PaV1, the transmission coefficient for contact transmission (0.115) is about an order of magnitude higher than that for waterborne transmission (0.026), suggesting that contact transmission may be a primary route of transmission by the virus in nature. However, since healthy lobsters avoid contact with diseased conspecifics and can do so well before the infected lobster becomes infectious (Behringer et al. 2006), the protection that host behavior affords in nature may modulate pathogen transmission by contact. Given the behavioral avoidance of diseased lobsters, our demonstration that lobsters are most vulnerable to infection during the asocial EBJ stage, and observations that the use of UV-treated water in recirculating culture systems vir-

tually eliminates PaV1 infection in EBJs (authors' unpubl. data) suggests that waterborne infection of small lobsters may be the most important mode of natural transmission.

Factors affecting susceptibility

The pathogenicity of many infections varies due to age- or size-specific changes in immune response, diet or habitat use that subsequently alter the demography of the population. For example, juvenile snow crabs *Chionoecetes oplio* have higher prevalences of infection by a parasitic dinoflagellate than do adults, which cease molting and are therefore no longer susceptible (Shields et al. 2005, 2007). Juvenile false king crabs *Paralomis granulosa* are susceptible to infection by a parasitic isopod, whereas adults are not, and this may be due to host size and the efficacy of gill cleaners in larger hosts (Roccatagliata & Lovrich 1999).

Transmission of PaV1 in *Panulirus argus* may too be affected by host (e.g. molt stage, condition, injuries) or environmental factors (e.g. water quality, temperature) that influence infection dynamics of other diseases in crustaceans (Shields 1992, Shields & Wood 1993, Messick & Shields 2000, Shields & Squyars 2000). For example, the decreased susceptibility of larger lobsters to PaV1 may be a consequence of ontogenetic differences in disease resistance. Alternatively, larger lobsters may benefit from a type of dose-response immunity and may be more capable of clearing a low dose of infectious virions from their systems. Perhaps the frequent molting of juvenile lobsters renders them more susceptible to pathogens that are most infectious at ecdysis. Maybe the vegetated, inshore nursery occupied by juveniles harbors prey or fomites with the virus that are not found on the coral reefs where adults dwell. As yet, we have answers to none of these questions.

Threats to wild populations, fisheries, and mariculture

Viral infections can have significant effects on natural populations of crustaceans subject to fishing or aquaculture. Pandemics of shrimp viruses have spread widely across the tropical and subtropical regions of the world with catastrophic results for penaeid shrimp aquaculture and fisheries. Whether PaV1 is an emerging pathogen or has simply gone unnoticed in the juvenile lobster population is not known. Anecdotal observations made during our studies of spiny lobster in Florida over more than 2 decades suggest that the disease has long been present, but perhaps at lower

prevalences than currently found. Its prevalence in the Florida Keys has not changed since we first discovered it in 1999, but PaV1 outbreaks in mariculture research facilities have become frequent and widespread, albeit infrequently reported. The prevalence of PaV1 infections in the greater Caribbean is as yet unknown, as is the risk of its spread; however, we have confirmed its presence in Florida, Belize, Mexico, and the Virgin Islands. The Caribbean spiny lobster sustains one of the most economically important fisheries in the Caribbean; thus, monitoring the prevalence of PaV1 among juvenile lobsters in nursery areas would seem prudent.

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