Appendix A: Supplemental Testing, Sensitivity, and Results

for the *Panulirus argus*-PaV1 Model

Contents

1. Model Fit to Observed Prevalence Values

To determine the most realistic scenario, we tested the effect of three levels of background infection: (a) no background infection rate, (b) a low 0.1% daily incidence derived from a 2.5% monthly incidence, and (c) a high 0.4% daily incidence derived from a 10% monthly incidence. In all of these simulations susceptible lobsters were allowed to detect and avoid infectious lobsters. We compared the results of these simulations to the mean of prevalence values observed at 12 sites in the Florida Keys over twelve years (Behringer et al. 2008, including data collected subsequent to publication) (Fig. A1). As shown, the low incidence level produced a trajectory with a mean of 9.9% that was within one standard error of the empirical mean. High EBJ incidence led to higher prevalence, averaging 1.5%, well above the empirical mean.

Typically, when fitting a model to an observed trajectory, a maximum likelihood approach using the observations is adopted. In this case, we did not do so for several reasons. First, the empirical data are based on visibly diseased lobsters, and are therefore an underestimate of true prevalence (Behringer et al. 2008). Second, the sites that were monitored were haphazardly chosen from sites that had been used for other purposes, eight of which had artificial shelters installed, the effects of which on disease dynamics are unknown; therefore the sites do not comprise a random sample of natural sites. Third, the observed time series covers the period from 2000 to 2012, whereas our simulations use population data from 1995-2005. Therefore, we have taken a semi-quantitative approach to evaluating the performance of the model.

2. Model Sensitivity to the Dose-Response Relationship

The dose-response function governs the probability that a susceptible lobster will contract disease when exposed to a given dose of virions. Equation A1, below, combines Equations 1 and 2 and simplifies the result to calculate the probability of infection, P_I:

$$P_I = 1 - (0.01 + m_s)^{\frac{\sum m_i}{\kappa m_s}} \quad \text{(A.1)}$$

In this equation m_i is the mass of an infectious lobster to which the focal susceptible lobster is exposed, m_s is the mass of the focal lobster, and κ is a constant of proportionality relating the biomass of a susceptible lobster to the effectiveness of a dose of virions produced by an infectious lobster of the same size. This function describes a surface that takes on different forms based on the value of κ (Fig. A2).

To test the sensitivity of the model to this function, we compared persistence (years to extinction) of PaV1 in the lobster population with κ = 1, which was also used for our main

comparisons, with results obtained with $\kappa = 1/75$. As shown by Fig. A2, panel B, the probability of infection is 1.0 for nearly every combination of susceptible and infectious lobster under the second scenario. This reduced the dose-response relationship to a nearly binary system in which dose no longer mattered. We reasoned that, if the results of these tests did not appreciably differ, then the model is not sensitive to the dose-response relationship. For these scenarios, there was no infection of EBJs, and disease avoidance began two weeks prior to infectiousness. To compare the results we used an independent samples t-test on natural log transformed data. Based on that test, we were unable to reject equality of the mean times to extinction (Table A1). The means differed by only 227 days, therefore we concluded that the model is not sensitive to the dose-response relationship. In view of this, we could have run all simulations with $\kappa = 1/75$; however, all simulations had already been run with $\kappa = 1$.

TABLE A1. Test of dose-response sensitivity. Means±1 standard error of untransformed data are shown.

$\kappa = 1$	$\kappa = 1/75$	df	t	P
2.15±0.58	2.77±0.53	18	0.925	0.367

3. Populating the Model

To determine the initial lobster population structure, 30 replicate runs of ten years were performed, with no initial population and no disease. The model was populated by simulating monthly influxes of postlarvae, which were distributed over the region and juvenile lobsters then allowed to grow, move, and die using the algorithms described herein. Annual recruitment was measured to find the year at which recruitment stabilized, which was considered the minimum

"spin-up" time necessary to produce a stable population. The cell-by-cell population structure of the model on January 1 of a randomly selected year after the spin-up period was used as the initial lobster population for all of the subsequent simulations.

After determining the initial lobster population for each cell, initial PaV1 prevalences were set to mimic observed prevalences. The initial prevalence for each habitat cell was randomly drawn from a discrete probability distribution function constructed from prevalences observed from June-August 2002 (Fig. A3). Although the data set used was larger (Butler unpublished data), data from sites with fewer than ten observations were excluded to avoid small sample size issues, such as 100% prevalence observed because only one lobster was caught and it happened to be infected.

4. Natural Mortality

Lobsters may potentially face mortality by predation while in their diurnal shelters, while foraging, and while searching for shelter or migrating. The probability of mortality for each lobster is calculated during each of those activities, with the exception being that algal-phase lobsters and those in seagrass cells do not search for shelter. The model assumes a diurnal period of 12 hrs, during which lobsters are quiescent in their shelters, and a two-hour crepuscular (dawn) period during which lobsters may search for a different shelter than that used during the previous day. The hourly probability of mortality (P_D) is computed as a function of lobster size (S) and shelter type:

$$P_D = P_O = 0.373 / 24 (0.305 + S) \quad \text{for lobsters in the open} \qquad (A.2)$$

$$PD = PS = 0.15 / 24 (-0.409 + S) \quad \text{for lobsters in macroalgae or crevice shelters} \qquad (A.3)$$

$$PD = Pg = 0.228 / 24 (-0.766 + S) \quad \text{for lobsters in seagrass} \qquad (A.4)$$

All lobsters in seagrass habitat cells remain in seagrass all of the time, so we use equation (A.4) for the day, twilight, and night periods (P_D = Pg). Algal-stage lobsters in hard-bottom cells remain in macroalgae all of the time, so we use Eq. A.3 for all three time-periods ($P_D = P_S$). The calculation is more complicated for postalgal-stage lobsters and for transitional lobsters in hard-bottom cells. Transitional and postalgal-stage juveniles are either in shelters (if available) or in sub-optimal shelters, assumed to have the same sheltering value as seagrass during the day (either Eq. A.3 or Eq. A.4). Lobsters ≤ 25 mm CL spend only one-third of the night foraging in the algae close to their dens, so Eq. A.4 is applied for 3.3 hours of their nightly activity, and Eq. A.3 for the balance of the night and for the two hours of crepuscular time. Larger juveniles spend variable amounts of twilight time in the open searching for shelter (Eq. A.2), and are in the seagrass at night (Eq. A.4). The fraction of twilight hours spent searching (T_P) is assumed to be inversely proportional to the availability of all appropriate shelters in that cell:

$$Tp = (K_T - N_t)/K_T \qquad (A.5)$$

where K_T is the total lobster carrying capacity of the cell (summed over shelter types that might be used by the lobster) and N_t is the current number of lobsters in those shelters. For these lobsters, Eq. A.2 was applied over $2T_P$ hours and Eq. A.3 for the remaining $2 - 2T_P$ hours. For each lobster searching for shelter, if a generated random deviate was less than P_D multiplied by the appropriate amount of time, the lobster was assumed to have died and was removed from the simulation.

Equations A.2 to A.4 were determined through least-squares fitting of equations to size-specific and shelter-specific mortality data derived from tethering studies (Smith and Herrnkind 1992). However, tethering data only yielded relative estimates of mortality among sizes and shelters, thus the appropriate intercepts for these functions were unknown. We estimated the

intercept terms in the equations using model simulations. Mark-recapture studies of microwire tagged first benthic stage juveniles out-planted in macroalgae indicate that only 1 to 4% survive to 35 mm CL (Butler *et al*. 1997, Sharp *et al* 2000). Therefore, we iteratively altered the intercept in Eq. A.3 and ran a single cohort of juveniles through the growth, shelter selection, and mortality routines with the constraint being lobsters only accessed hard-bottom habitat where they could move between macroalgae and hard-bottom structures of unlimited lobster carrying capacities. From these simulations, we chose a mortality function for lobsters dwelling in hard-bottom cells that resulted in 1 to 4% of the model individuals surviving to 35 mm CL. Based on tethering results, we then assumed that survival of lobsters in open water was one-half that of those in hard-bottom cells, and so selected an intercept for Eq. A.2 that resulted in 0.5 to 2% survival to 35 mm CL. Tethering studies also suggest that survival of juveniles in seagrass falls midway between that measured in the open and in hard-bottom structures or macroalgae (Herrnkind and Butler 1986). We therefore chose an intercept for Eq. A.4 (lobsters in seagrass) that resulted in survival intermediate to the survival in the open and in hard-bottom cells. In addition, early benthic juveniles (≤ 10 mm CL) are subject to mortality as a function of salinity (S_L) and temperature (T). Weekly survivorship (L) is given by the function:

$$L = 1.236 \times \exp\left\{-0.5\left[\left(\frac{T - 24.39}{5.565}\right)^2 + \left(\frac{S_L - 35.31}{8.587}\right)^2\right]\right\} \qquad \text{(A.6)}$$

The daily probability of mortality is then given by $P_D = 1 - e^{L/7}$.

5. Notes on Simulation Replication and Power

The model used for this research was very computationally intensive. Each set of replicated runs required more than a day to run, involved simulations of millions of individual

lobsters, and generated more than 200 Mb of output data each. Therefore, it was not possible to generate thousands of replicate runs as is typically done for more simplistic approaches. Instead, we used the same sampling and analytical approach that is typically used for experimental manipulations of complex natural systems. That is, we tested a few, specific values for the factors of interest and analyzed the results using ANOVA.

Each simulation consisted of ten years to ensure that long-term trends could be observed. The number of replicate runs needed to detect a 5% change in total lobster recruitment to the 50 mm CL size class was determined using a Visual Jackknife technique (Confalonieri et al. 2007). The results of twenty replicate runs that simulated no disease effects and which used the default temperature function, uniform oceanic salinity of 35 psu, and no cyanobacteria blooms, were analyzed. The optimal sample size was determined by resampling the data, systematically taking larger subsamples to find the subsample size at which the rate of change of the subsample means was negligible. For each subsample size, 500 sets of subsamples were drawn to determine the mean and variance, resulting in the Visual Jackknife optimal sample size of six replicates. The minimum sample size necessary to detect a 1% difference from the mean at the $\alpha=0.05$ level was also calculated using an iterative procedure based on Student's T, with identical result. Because the variability of the system under other scenarios was not known, the sample size estimate was rounded upward to the nearest ten to ensure sufficient replication.

6. Literature Cited

Behringer, D. C., Jr., M. J. Butler, IV, and J. D. Shields. 2008. Ecological and physiological effects of PaV1 infection on the Caribbean spiny lobster (*Panulirus argus* Latreille). Journal of Experimental Marine Biology and Ecology 359:26–33.

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Sharp, W. C., W. A. Lellis, M. J. Butler, IV, W. F. Herrnkind, J. H. Hunt, M. Pardee-Woodring, and T. R. Matthews. 2000. The use of coded microwire tags in mark-recapture studies of juvenile Caribbean spiny lobster, *Panulirus argus*. Journal of Crustacean Biology 20:510–521.

Smith, K. N., and W. F. Herrnkind. 1992. Predation on early juvenile spiny lobsters *Panulirus argus* (Latreille): Influence of size and shelter. Journal of Experimental Marine Biology and Ecology 157:3–18.

7. ANOVA Tables

TABLE A2. Three-factor model I ANOVA testing the effects of Disease Avoidance Timing × Density-Independent Infection of Early Benthic Phase Lobster × Presence of Exogenous Infection of Postlarvae on the simulated lobster recruitment. Here, recruitment is the number of lobsters surviving to 50 mm CL. There is a significant three-way interaction among the factors.

Source	df	MS	*F*	*P*
Density-Independent EBJ Infection (EBJs Infected)	1	165379.60	185.83	0.0009
Exogenous Infection of Postlarvae (PLs Infected)	1	39187.60	7.21	0.3441
Disease Avoidance (Avoidance)	3	37705.62	42.37	0.0059
EBJs Infected × PLs Infected	1	6916.90	3.11	0.1759
EBJs Infected × Avoidance	6	889.95	24.84	<0.0001
PLs Infected × Avoidance	3	741.75	0.33	0.8041
EBJs Infected × PLs Infected × Avoidance	3	2221.92	62.03	<0.0001
error	144	35.82		
Total	162			

TABLE A3. Two-factor model I ANOVA testing the effects of disease avoidance (Avoid) and HABs (HAB) on lobster recruitment in Florida Bay. There were three levels of disease avoidance, based on the timing of the onset of the behavior relative to when exposed conspecifics become infectious: never, two weeks before, and four weeks before. There were two levels of HAB, present and absent. Multiple comparisons (REGWF Test) were performed following the significant ANOVA. Homogenous subsets are indicated by a solid line beneath the mean values.

Source	df	MS	*F*	*P*
Avoid	2	0.297	46.673	<0.0005
HAB	1	10.810	1701	<0.0005
Avoid×HAB	2	0.005	0.0776	0.463
Error	84	0.006		
Total	89			

R-E-G-W-F Test on disease avoidance:

Treatment	No Avoidance	Two Weeks	Four Weeks
Mean Recruitment	13898	16889	16951

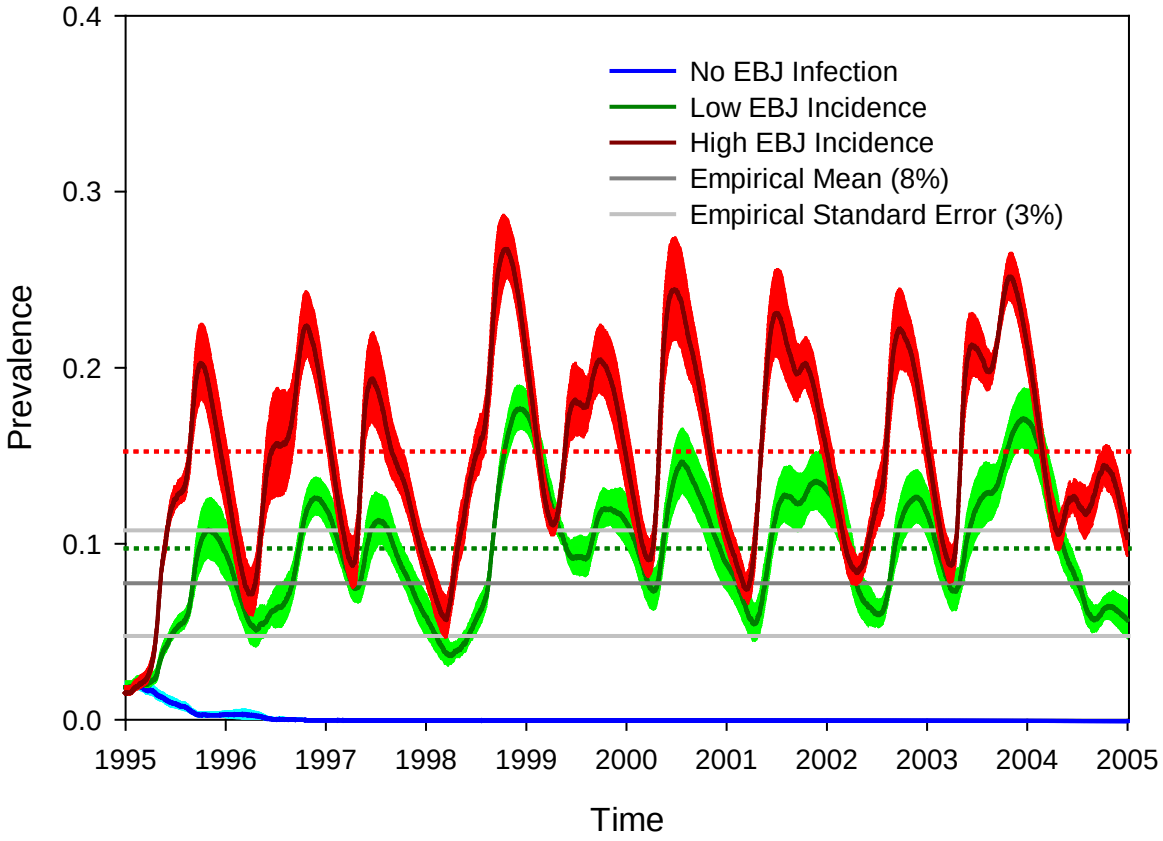
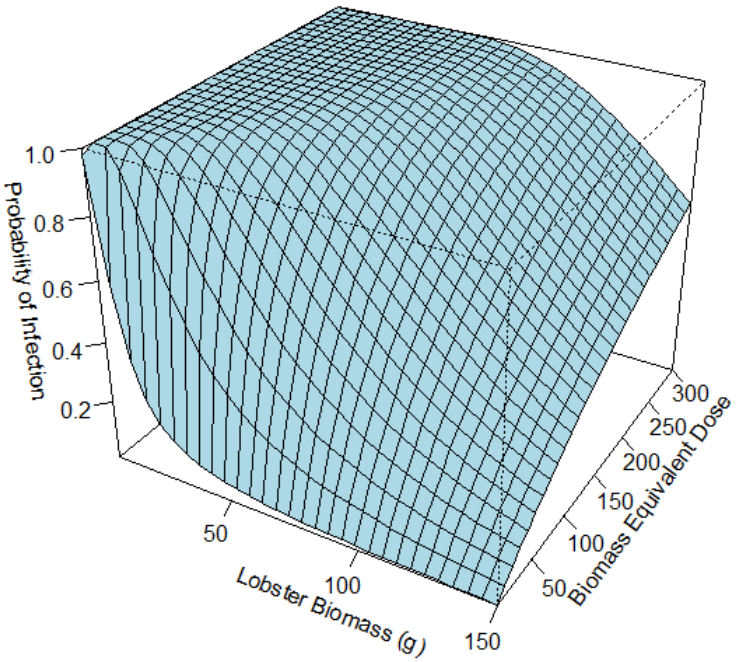


FIG. A1. Prevalence of PaV1 for different models of transmission to early benthic juvenile lobsters (EBJs). The observed mean and standard error of visibly detectable PaV1 prevalence at twelve sites is indicated by the solid gray lines. Note that these empirical estimates based on visibly diseased lobsters are underestimates of all lobster infected by PaV1, which is what the model predicts. The means of simulations using high or low incidence values are indicated by dotted lines that are the same color as the trajectory of prevalence over time. The standard deviation for each scenario is indicated by the width of the color bands around each trajectory line. The mean prevalence under the low incidence scenario falls within one standard error of the empirical values, suggesting that this provides a better fit that the high incidence scenario.


Probability of Infection
1.0
0.8
0.6
0.4
0.2
50
100
150
Lobster Biomass (g)
50
100
150
200
250
300
Biomass Equivalent Dose

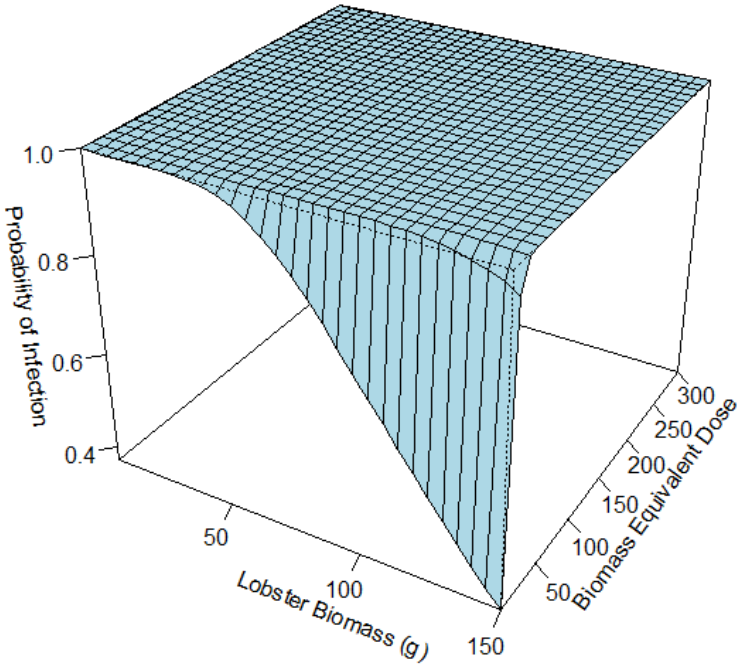

Probability of Infection
1.0
0.8
0.6
0.4
50
100
150
Lobster Biomass (g)
50
100
150
200
250
300
Biomass Equivalent Dose

FIG. A2. Probability of PaV1 transmission by infectious lobster size and susceptible lobster size. Generally, susceptibility to PaV1 is higher for smaller lobster. The dose of virions received by the susceptible lobster is based on the cumulative mass of all infectious lobsters to which it is exposed. The “equivalent infectious lobster size” is the size of an individual lobster that would produce the same dose of virions as that same mass composed of several smaller lobster. Also, note that, although lobsters less than 15 mm CL are shown on the graph, very few of those would be in crevice shelters, thus would neither be exposed nor contribute to the exposure of other lobster by this mechanism. Panel A shows the form of the function as it was used for our main comparisons ($\kappa = 1$). Panel B shows the function with the dose-response maximized ($\kappa = 1/75$).

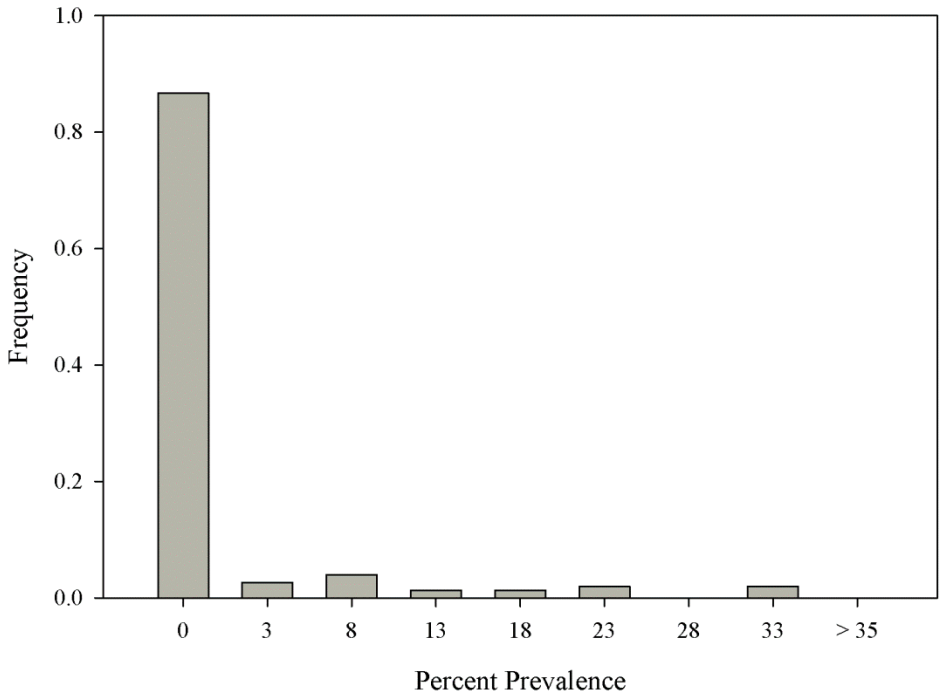


FIG. A3. Frequency distribution of 150 observed PaV1 prevalence values in the Florida Keys from June-August 2002. Values have been combined in five percent intervals except for 0% and > 35%. The values shown on the x-axis are the midpoints of the intervals. In the model the actual (randomly selected) observed percentages were applied to each cohort of newly arriving lobster.

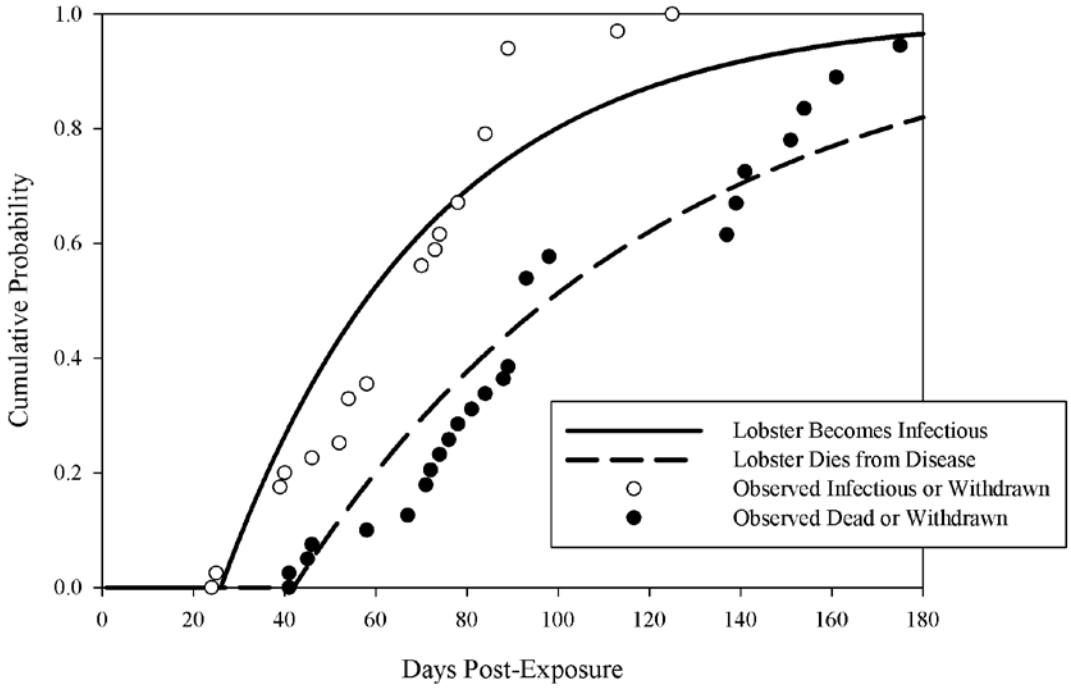


FIG. A4. Cumulative probability of becoming infectious and of disease-induced mortality with time. Lines are calculated cumulative probabilities based on two-parameter exponential survival functions. Plotted points are the observed cumulative proportions of individuals that became symptomatic of PaV1 infection (open circles) and those that died (filled circles) of a group of 40 experimentally infected individuals. The observations also include censored observations (withdrawn from the study before a state change was observed).

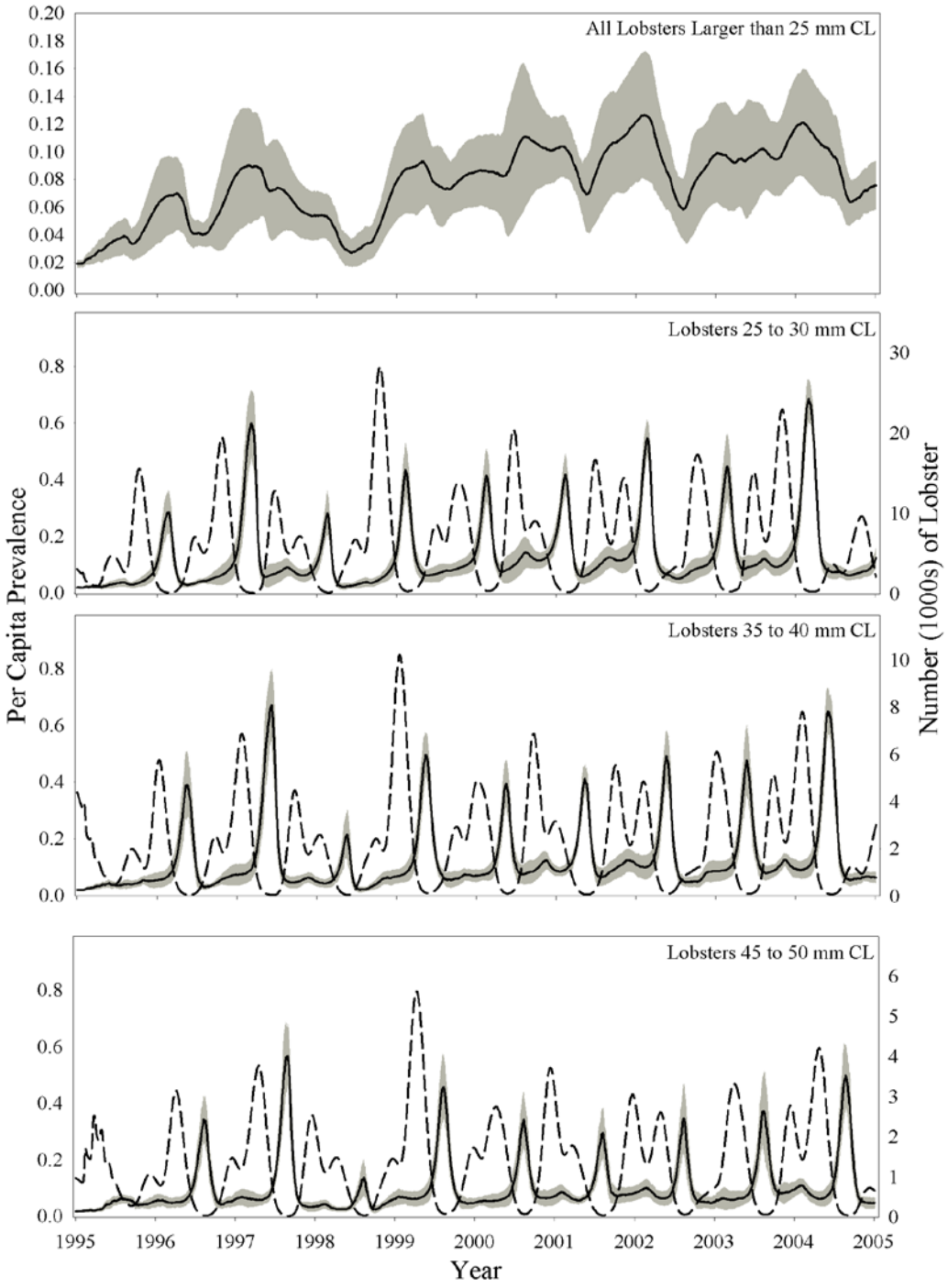


FIG. A5. Decomposition by size class of prevalence in lobsters > 25 mm CL. Dashed lines are lobster abundances (right y-axis of each graph). Solid lines are the mean daily prevalence for ten

simulations ±1 standard error. Annual peak values in prevalence occur as the number of lobsters in each size class decreases due to growth of healthy lobsters while infected lobsters fail to grow.