ELSEVIER

Journal of Experimental Marine Biology and Ecology 359 (2008) 26–33

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Ecological and physiological effects of PaV1 infection on the Caribbean spiny lobster (*Panulirus argus* Latreille)

Donald C. Behringer [a,*], Mark J. Butler IV [b], Jeffrey D. Shields [c]

[a] *Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32653 USA*
[b] *Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529 USA*
[c] *Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA*

Received 29 November 2007; received in revised form 13 February 2008; accepted 14 February 2008

Abstract

Pathogens can profoundly impact the ecology of the organisms they infect through changes in host behavior that influence demographic processes. For example, juvenile Caribbean spiny lobsters (*Panulirus argus* Latreille) infected with the PaV1 virus (*Panulirus argus* Virus 1) are avoided by their normally social conspecifics, which alters local spatial distributions and presumably rates of disease transmission. PaV1 infections are nearly always lethal, but prior to succumbing to the disease, infection may impact other host dynamics (e.g., movement, growth, or survival) that effect transmission of the virus. We used mark-recapture surveys and laboratory studies to determine the impact of PaV1 infection on lobster movement and physiological condition, and in turn, the effect of condition on susceptibility to infection. Significantly more healthy lobsters were recaptured than heavily diseased lobsters in 5-d mark-recapture surveys, indicating either greater emigration or greater mortality of infected lobsters. Results of a laboratory bioassay suggest that lobsters with early-stage infections moved at an equivalent rate to healthy lobsters, however, as infection progressed, lobsters inoculated with PaV1 moved less than healthy lobsters ultimately remaining sedentary. Infected lobsters captured in nature also had significantly lower hemolymph (blood) serum protein values, indicating poor physiological condition. However, poor condition did not predispose lobsters to greater risk of infection because prevalence was not different between starved lobsters and healthy lobsters challenged with PaV1. Although lobsters infected with PaV1 eventually become sedentary and of poor nutritional condition, during the early stages of infection they remain active and are thus capable of dispersing the virus throughout the population.

Keywords: Crustacea; disease; ecology; movement; nutritional condition; susceptibility; virus.

1. Introduction

There are extraordinary examples of parasite-mediated changes in host behavior (e.g., Hamilton and Zuk, 1982; Moore, 1983; Curtis 1987; Goulson, 1997; Vyas et al., 2007), but the behavioral response of hosts infected by bacterial or viral pathogens is often non-specific, and includes depression and lethargy. In rare instances, behavioral modification by bacterial or viral infections can be spectacular, as is the case with the rabies virus in mammals (MacDonald, 1980; Hart, 1988, 1990). More commonly, host activity decreases in response to bacterial or viral infection, and this morbidity may decrease transmission of the pathogen. It may also reduce contact among infected hosts (Loehle, 1995; Packer et al., 2003) allowing them to preserve energetic resources and resist or eliminate the pathogen (see Hart, 1988 for review). Increased lethargy may also result in higher predation that culls sick animals from the population, which removes the pathogens as well (Lefcort and Blaustein, 1995).

PaV1 is the first naturally occurring, pathogenic virus known to infect lobsters. One prominent sign of PaV1 infection is lethargy in the spiny lobster host, presumably due to metabolic wasting and tissue ischemia (Shields and Behringer, 2004). In nature, PaV1 infects juvenile Caribbean spiny lobsters, *Panulirus argus* (Shields and Behringer, 2004) and although the virus has

* Corresponding author. Tel.: +352 392 9617x284; fax: +352 392 3672.
E-mail address: behringer@ufl.edu (D.C. Behringer).

0022-0981/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2008.02.012

not been fully characterized (Montgomery-Fullerton et al., 2007), it is a non-enveloped, icosahedral virus that replicates entirely within the nucleus (Shields and Behringer, 2004).

Lobsters infected by PaV1 are avoided by healthy lobsters; a behavior that presumably reduces the risk of infection for uninfected hosts (Behringer et al., 2006). This action is contrary to the normal gregarious nature of *P. argus* and results in isolation of infected individuals. Although avoidance behavior such as this is rare in nature, all organisms maintain defenses to the invasion of pathogens. These defenses include hygienic behaviors (e.g., grooming, preening, bathing, and den sanitation), physical barriers (e.g., skin, exoskeleton, mucous), and physiological defenses (e.g., humoral and immune defenses, non-self recognition, phagocytosis, encapsulation, and nodulation).

For crustaceans, humoral defenses include circulating antimicrobial peptides such as bactericidins, agglutinins, and opsonins (Bachère et al., 2004). Their cellular defenses include phagocytic and encapsulating activity of hemocytes, induction of the phenoloxidase pathway, and infiltration by hemocytes to a site of injury or infection (see Shields et al., 2006 for review). These defenses, though normally formidable, may become compromised in the presence of physiological stress brought on by disease (Brock and Lightner, 1990). Stress can act to weaken the immune system allowing invasion and infection by external pathogens or multiplication of opportunistic agents. In the case of PaV1 disease, the virus can be transmitted among lobsters via inoculation, prolonged contact with infected lobsters, ingestion of infected tissue, and over short distances in the water (Butler et al., in press). Although small lobsters are more susceptible to PaV1 infection than large lobsters (Butler et al., in press), conceivably due to ontogenetic differences in immune function, the effect of stress on the risk of PaV1 infection is unknown. For example, stress associated with poor nutritional condition, such as that experienced by juvenile lobsters that are confined for long periods in fishing traps as live attractants, potentially increases their risk of PaV1 infection.

Our objectives were: (1) to examine the effect of PaV1 infection on patterns of residency among lobsters in the field, (2) to determine how infection affects lobster movement in the laboratory, (3) to investigate the relationship between PaV1 infection and physiological condition among lobsters in the field, and (4) to asses the impact of physiological condition on susceptibility to infection by PaV1.

2. Materials and methods

2.1. Ecological effects

2.1.1. The effect of infection on lobster movement

To assess the effect of PaV1 infection on the movement of naturally infected lobsters, we used a mark-recapture study to estimate the short-term residency of infected and uninfected animals. Mark-recapture experiments were performed over a five-day period during each survey in the summer of 2000, winter of 2001, and the summer of 2001 at 12 sites (9 in summer 2000) on the bayside of the middle and lower Florida Keys, USA (Fig. 1). Site locations were chosen haphazardly within the hard-bottom nursery areas available in the region. Hard-bottom habitat is found throughout the shallow waters surrounding the Florida Keys and comprises 30 – 40% of the available bottom <3 m deep (Zieman et al., 1989; Herrnkind et al., 1997). Each site was a 25 m × 25 m area delineated with polypropylene rope attached at four corners to concrete blocks. Sites ranged in depth from 1 - 3 m and were 1 - 7 km from shore.

On the initial day of each mark-recapture period, one or two SCUBA divers searched the entire site, captured each lobster, and recorded the carapace length (CL), sex, molt condition (pre-molt, inter-molt, or post-molt), and the disease status (diseased or healthy) of each lobster. The lobster was then marked with a unique color-banded antenna tag and returned to its den. Five days later, the divers searched the entire site again, captured all of the lobsters encountered, and recorded their tag codes. The lobsters were then brought onboard the research vessel where

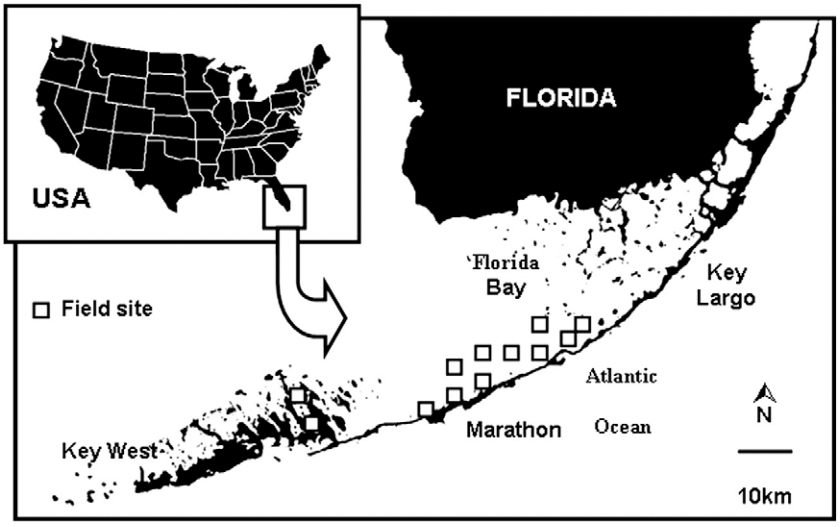

Fig. 1. Location of the field sites (squares) in the Florida Keys (USA) where disease prevalence was monitored and where mark-recapture studies were conducted.

they were assessed for visible signs of disease. Discolored (i.e., chalky or milky white) hemolymph was used as evidence of infection and was viewed via inspection of the dorsal juncture between the cephalothorax and the abdomen. Visibly infected lobsters were transported back to the laboratory, anesthetized on ice for 15 min, then their heart, gills, hepatopancreas, epidermis, hindgut, and abdominal muscle tissues were dissected and fixed in 10% neutral-buffered formalin for histological determination of infection level as in Li et al. (2008; 0=healthy; 1=light infection; 2=moderate infection; 3=heavy infection). The impact of PaV1 infection on our recapture success of healthy and diseased lobsters was evaluated using a 3-way log-linear model (survey x health status x recapture status). To ensure that the tagging procedure did not agitate the lobsters and cause disproportionate emigration during any of the surveys, we analyzed the number of lobsters on each site during day 1 and day 5 using a 2×3 model-1 ANOVA where the factors were mark-recapture day and survey period.

2.1.2. Laboratory movement assay

To determine the effect of PaV1 on rates of movement by juvenile lobsters during the course of infection, we designed a laboratory raceway in which we measured the activity of healthy lobsters and lobsters infected with PaV1. In November 2002, we used 1-cc tuberculin syringes with 27-ga. needles to inoculate 21 juvenile lobsters (35 - 50 mm CL) with 0.3 ml of hemolymph from a PaV1 infected donor; for "healthy" controls; ten other lobsters were inoculated with hemolymph from a healthy lobster. The inoculum was injected through the arthrodial membrane between the basis and ischium of the 5th periopod. Lobsters were then held in isolation in 44-L flow-through aquaria in the laboratory, under natural photoperiod, and fed squid and shrimp ad libitum daily. Visible signs of PaV1 infection typically occur 6 – 8 weeks post-inoculation (Behringer et al., 2006), so control and infected lobsters were assessed with the movement bioassay (described below) in January and February 2003 (i.e., 2 – 3 months after inoculation).

Four raceways were used in the lobster movement bioassay, requiring several days to test all of the lobsters. The raceways were constructed using round (2 m wide x 1 m deep) plastic tanks into the center of which we installed a round barrier, creating a circular track ~6 m long by 0.5 m wide (Fig. 2). Each raceway was supplied with aerated, flow-through seawater and a single PVC shelter for use by the lobster. An event recorder was positioned at the opposite side of the raceway from the shelter. The event recorder consisted of an array of five clear plastic rods (5 mm diameter) loosely suspended across the raceway just above the bottom and attached to mercury switches that triggered a HOBO© event recorder (Onset Equipment Inc.) whenever the rods were moved by a passing lobster. In preliminary tests, lobsters were unaffected by the rods and did not hesitate to push through them. We began each trial by introducing a single lobster into a raceway at approximately 5:00 pm then removing it two days later. Each trial was conducted under natural photoperiod. We recorded the water temperature at the beginning and end of each trial because water temperature affects metabolic rate and, thus, lobster activity. The mean number of movement "events" per day was used in a 2×2 repeated-measures MANOVA to test the effect of PaV1

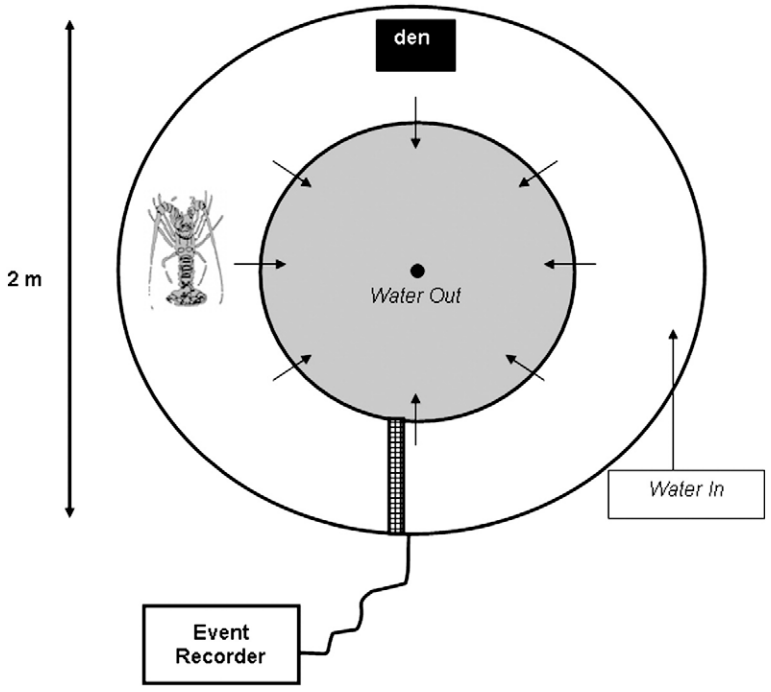

Fig. 2. Raceway design for the lobster movement assay in which we measured the frequency of movement of PaV1 infected and healthy lobsters past an event recorder during 2d trials in January and February 2003. Arrows indicate the general flow of seawater from the external flow-through source, into the raceway, and out through the center barrier and standpipe.

infection and development time (i.e., month) on relative rates of movement by lobsters.

2.2. Physiological effects

2.2.1. Effect of PaV1 infection on physiological condition

To determine if physiological condition differed between healthy lobsters and those infected with PaV1, we measured the hemolymph refractive index of a subset of the lobsters captured in the mark-recapture study described above. The virus was initially discovered mid-way through a separate study on physiological condition in 1999, so these data were included in this analysis. A haphazard subset of 20 healthy lobsters (or all if less than 20 were present on the site), and any visibly infected lobsters, were selected for measurement of their hemolymph refractive index. This index provides an estimate of hemolymph serum protein concentration, which is a proxy for physiological condition once molt stage is taken into consideration (Musgrove, 2001; Oliver and MacDairmid, 2001; Shields et al., 2003; Behringer and Butler, 2006). Serum protein levels vary with molt stage (Smith and Dall, 1982; Depledge and Bjerregaard, 1989), therefore, we used the method described by Lyle and MacDonald (1983) to determine the molt stage of each lobster captured at each site on the final day of the mark-recapture period. A 2×4 model-I ANCOVA was used to determine if PaV1 infection had a significant impact on the mean hemolymph refractive index.

2.2.2. Effect of physiological condition on PaV1 susceptibility

To test the hypothesis that physiological stress similar to that experienced by trap-confined lobsters increased the susceptibility of lobsters to PaV1, we held large (50 - 65 mm CL) juvenile lobsters in individual flow-through ambient seawater tanks (44-liter capacity) and fed them a diet of squid and shrimp at two ration levels; 100% (n=14) and 25% (n=10) of their predetermined maximum daily consumption. The disparity in these ration levels is known to result in significantly different physiological conditions after four weeks as measured by the hemolymph refractive index (Behringer and Butler, 2006). Lobsters were fed at these ration levels for four weeks to ensure that they differed in condition prior to being challenged with PaV1. Lobsters were then fed 1 g of muscle tissue from an infected donor once per week. After 75 d, the hemolymph refractive index of each lobster was measured again and the experiment terminated. The lobsters were then anesthetized and tissues prepared for histological examination as described above. A 2×2 repeated-measures MANCOVA was used to confirm whether lobsters in the two food ration treatments differed in blood protein concentration (i.e., physiological condition) at the initiation and termination of the experiment. A Fisher Exact test was used to evaluate whether susceptibility of lobsters to PaV1 was independent of physiological condition.

Table 1
A 2×3 model-I ANOVA comparing the number of lobsters on sites surveyed on day 1 and day 5 of mark-recapture studies during survey periods: Summer 2000, Winter 2001, and Summer 2001

Source	df	SS	F	P
Survey period	2	1006.4	2.01	0.143
Survey day	1	8.450	0.0337	0.855
Survey period x Survey day	2	103.20	0.206	0.814
Error	60	1.5×10^4		

Table 2
A three-way log-linear analysis of the difference in the short-term residency between visibly infected and visibly non-infected lobsters on sites where mark-recapture studies were conducted during survey periods: Winter 2001 and Summer 2001

Effects	df	Likelihood ratio X^2 change	P
Survey period x Health status x Recapture status	1	0.067	0.796
Survey period x Health status	1	0.493	0.483
Survey period x Recapture status	1	0.094	0.759
Health status x Recapture status	1	5.909	0.015*

* significance determined at alpha=0.05

3. Results

3.1. Ecological effects

3.1.1. Effect of infection on lobster movement

The total number of lobsters on each site remained consistent from day 1 to day 5 for each survey (Table 1), indicating that our survey procedures did not elicit emigration from the sites. We recognize that survey periods were not independent because many of the same sites were included in each survey. However, we explicitly accounted for survey period in the statistical analysis; furthermore, the growth and movement of lobsters relative to the time elapsed between surveys makes it highly unlikely that any lobsters would have been surveyed twice (i.e., non-independent). A repeated-measures ANOVA was not used in the analysis because all sites were not repeatedly measured during each survey.

Inspection of the data revealed that too few diseased lobsters were marked (n=6) during Summer 2000 to provide a meaningful comparison, so that sample period was removed

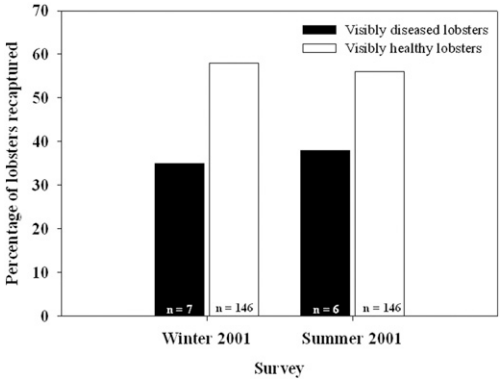

Fig. 3. The percentage of visibly diseased and healthy lobsters recaptured during 5d mark-recapture surveys conducted in the winter 2001 and summer 2001. The n-values represent the number of lobsters recaptured during each survey.

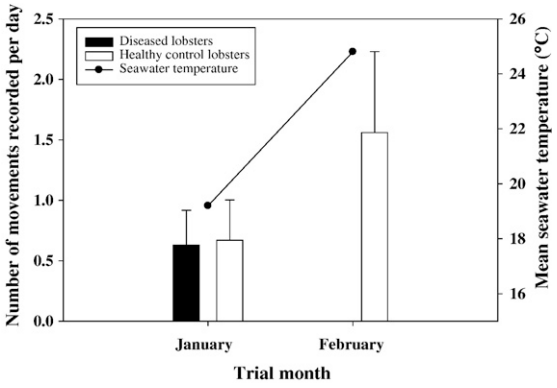

Fig. 4. The relationship between daily movement and infection by month for lobsters inoculated with PaV1-infected hemolymph (treatment) or with uninfected hemolymph (control). Lobsters were tested over 2d each month (January – February) in laboratory raceways. Ambient seawater temperature is noted due to its impact on lobster metabolism and movement. Error bars represent 1 SE.

from further analysis. Analysis of the remaining surveys showed a significant positive relationship between health status and proportion of animals recaptured, with greater recaptures among healthy lobsters compared to diseased animals (Table 2; Fig. 3).

3.2. Laboratory movement assay

Three months after inoculation, lobsters infected by PaV1 were significantly less active than healthy lobsters despite background changes in temperature that also influenced their movement (Fig. 4; MANOVA, Table 3). In January 2003, several successive cold fronts lowered the mean ambient seawater temperature in our experimental raceways to 19.2 °C. This low temperature depressed the movement of all lobsters regardless of treatment (Fig. 4). The mean temperature rose to 24.8 °C in February and the control lobsters responded with a concomitant increase in movement, whereas the activity of diseased lobsters remained low (Fig. 4; MANOVA, Table 3). The interaction between month and health status ($P=0.064$), and the impact of health status alone ($P=0.052$) were both marginally significant, probably due to the effect of low seawater temperature in depressing movement in general. Mortality of diseased lobsters was high following the February trial, which precluded additional raceway trials.

Table 3
Results of a 2×2 repeated-measures MANOVA examining the effect of PaV1 infection on the number of lobster movements during 2 d trials in laboratory raceway experiments conducted in January – February 2003

Source	Numerator df	Denominator df	F	P
Between Subjects				
Health treatment (A)	1	25	4.155	0.052
Within Subjects				
Raceway trial (B)	1	25	0.072	0.791
A x B Interaction	1	25	3.762	0.064

Table 4
Results of a 2×3 model-I ANOVA examining the impact of PaV1 infection on the mean hemolymph refractive index of lobsters collected during survey periods: Summer 2000, Winter 2001, and Summer 2001

Source	df	SS	F	P
Health status	1	564.9	28.23	<0.001*
Survey period	2	11.24	0.281	0.755
Health status * Survey period	2	12.04	0.301	0.740
Error	496	9927.5		

*significance determined at alpha=0.05

3.3. Physiological effects

3.3.1. Effect of PaV1 infection on physiological condition

The physiological condition (i.e., hemolymph refractive index) of lobsters visibly infected with PaV1 was significantly lower than that of visibly healthy lobsters at the end of each 5 d mark-recapture survey (Table 4; Fig. 5). Although hemolymph refractive index has been demonstrated to vary with molt stage (Behringer and Butler, 2006; and references therein), it was not included as a covariate in this analysis because lobsters infected with PaV1 were exclusively in the intermolt stage and we therefore used only healthy lobsters in intermolt stage for comparison. The effect of infection on hemolymph proteins was similar among survey dates, with neither the survey date nor the interaction between health status and survey date significant in the analysis.

3.3.2. Effect of physiological condition on PaV1 susceptibility

The physiological condition of lobsters in the 100% feeding treatment ($n=14$) was significantly greater than the group fed the 25% ration ($n=10$) at the beginning of the transmission trial and at the termination of the trial (Table 5). The analysis also revealed a significant effect of sampling time, as the hemolymph refractive index declined in both treatments over the course of the study. The non-significant interaction between sampling time and feeding treatment showed that the treatments were different and remained so throughout the experiment. However, after the 75 d trial period there was no significant difference between treatment groups in the number of lobsters

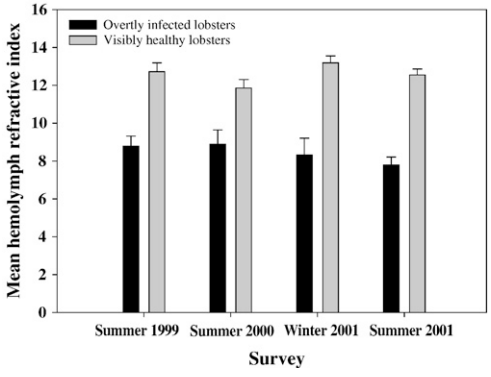

Fig. 5. The mean hemolymph refractive index (RI) for visibly diseased and healthy lobsters captured on the last day of 5 d mark-recapture surveys conducted 1999 – 2001. Error bars represent 1 SE.

Table 5
Results of a 2×2 repeated-measures MANOVA examining the nutritional condition (hemolymph refractive index) of lobsters at the beginning and end of a 75 d experiment investigating the effect of nutritional condition on PaV1 susceptibility

Source	Numerator df	Denominator df	F	P
Between Subjects				
Feeding treatment (A)	1	22	67.574	<0.0001*
Within Subjects				
Sample time (B)	1	22	11.95	0.002*
A x B Interaction	1	22	1.043	0.318

* significance determined at alpha=0.05.

infected with PaV1 (Pearson $X^2=0.137$, $P=0.71$); however, in both treatments only two lobsters became infected.

4. Discussion

PaV1 is an omnipresent threat to juvenile Caribbean spiny lobsters, impacting both their ecology and physiology. We have shown that PaV1 decreased the short-term residency of heavily infected individuals on field sites, undoubtedly due to lower survival and not higher emigration, as our laboratory movement assays confirmed that lobsters become lethargic and die as their infections progress. However, in the early stages of infection movement rates of infected and healthy lobsters were similar, so diseased lobsters continue to disperse throughout the population during this period. Lobsters infected with PaV1 also had a poorer nutritional condition relative to healthy conspecifics. However, their poor nutritional condition did not increase their susceptibility to PaV1 infection.

4.1. Ecological effects

The ecological impacts of disease are often subtle and difficult to measure, especially in cryptic host-pathogen relationships where it can be hard to document ecological change. Our laboratory movement assay results, although confounded by the low seawater temperatures that prevailed during that experiment, suggest that lobsters in the early stages of PaV1 infection are just as active as uninfected lobsters and probably move among areas at similar rates. Although not infectious until 6 – 8 weeks after contracting the disease (Behringer et al., 2006), the movement of eventually infectious individuals into new areas still promotes the spread of the pathogen. However, this dispersal of infected individuals may be partially offset by the ability of healthy lobsters to detect and avoid these individuals even before they become infectious (Behringer et al., 2006).

The sedentary behavior of lobsters with overt PaV1 infections initially led us to believe that we would observe significantly greater recapture rates among this group; yet, the opposite was true. Fewer infected lobsters were recaptured relative to healthy lobsters, presumably because of mortality. Clearly this result is not a consequence of increased emigration by heavily diseased lobsters. The laboratory movement assay performed on infected and healthy lobsters confirms that lobsters infected with PaV1 are less active once infection fully develops, usually a few months after contraction of the virus. These experimental results confirm our initial observations (Shields and Behringer, 2004; Behringer et al., 2006) that infected lobsters in the wild, which are often encrusted with fouling organisms (e.g., barnacles, epiphytic algae, and worm tubes) and coated with a layer of silt, are sedentary and have ceased grooming. Thus, heavily diseased lobsters are unlikely to spread the disease to new areas.

The lower recapture of diseased lobsters relative to healthy lobsters is most simply explained by the disease-induced death of lobsters in the late stages of PaV1 infection. However, we suspect that this was not always the case during the short duration of our mark-recapture studies. As PaV1 infection progresses and lobsters become lethargic and are shunned by their normally social conspecifics, they may be preyed upon more easily; thus accounting for their lower numbers in our recapture surveys. For spiny lobsters, the avoidance of PaV1 infected lobsters by healthy ones (Behringer et al., 2006) denies diseased lobsters the benefit of gregariousness and group defense from predators (Eggleston and Lipcius, 1992; Mintz et al., 1994; Butler et al., 1997; Dolan and Butler, 2006). Diseased animals are often at an increased risk of predation (Duffy et al., 2005; Johnson et al., 2006), which is the result of increased physical or behavioral conspicuousness, depressed anti-predatory behavior, or morbidity. For pathogens, the typical benefit is enhanced transmission, especially if it requires an intermediate host for completion of its life cycle (see Holmes and Bethel, 1972; Dobson, 1988; Poulin, 1995 for reviews). However, changes in host behavior that increase the susceptibility of the host to predation in the absence of intermediate hosts are often a by-product of a non-specific response to infection or stress (Horton and Moore, 1993; Lefcort and Blaustein, 1995; Poulin, 1995). The pathogen defense response, termed the acute phase response, includes symptoms such as fever (physiological or behavioral), lethargy, and malaise (Lefcort and Eiger, 1993). Although hypothesized to enable an organism to fight an infection by inhibiting the pathogen (Hart, 1988, 1990; Lefcort and Eiger, 1993), the acute phase response behavior may increase mortality by increasing the susceptibility of the host to predation. Lefcort and Eiger (1993) used alcohol-killed bacteria (*Aeromonas hydrophila* Chester) to elicit normal pathogen defense responses in bullfrog tadpoles (*Rana catesbeiana* Shaw) and found that those responses enhanced predation by the roughskin newt (*Taricha granulose* Skilton). Similarly, Lefcort and Blaustein (1995) used a directly transmitted yeast parasite (*Candida humicola* Knüsel) to elicit behavioral alterations in red-legged frog tadpoles (*Rana aurora* Baird and Girard) that also increased predation by *T. granulosa*. In these examples, the pathogen did not benefit from consumption of the host; on the contrary, increased predation of the compromised host by predators that are immune to infection should reduce pathogen transmission if sufficient numbers of infected hosts are culled from the population.

4.2. Physiological effects

The notable lethargy associated with advanced PaV1 infection is probably due to the synergistic effect of the depletion of energy reserves (i.e., glycogen in reserve inclusions), ischemia (Shields

and Behringer, 2004), changes in their metabolic state (Li et al., 2008), depressed physiological condition, and decreased feeding activity. During all surveys, infected lobsters had consistently lower hemolymph protein levels and, therefore, reduced nutritional condition. The poor condition of infected individuals may result, in part, from reduced foraging. Indeed, infected lobsters held in the laboratory gradually cease feeding, in correspondence with disease progression.

Although depressed nutritional condition is an indicator of metabolic exhaustion, it alone does not appear to increases the susceptibility of juvenile lobsters to infection with PaV1. Similarly, Braid et al. (2005) found that starvation did not increase the susceptibility of red abalone (*Haliotis rufescens* Swainson) to "*Candidatus* Xenohaliotis californiensis", the causative agent of withering syndrome. However, once infected, starvation brought about by changes in the digestive tubules in abalones may act synergistically with infection to accelerate the signs of disease, as may be the case for PaV1 infected lobsters.

4.3. Conclusions

PaV1 clearly affects the ecology and physiology of juvenile lobsters in the field, including altered behaviors (Behringer et al., 2006), changes in movement rates, decreased physiological health, and lower survivorship (Butler et al., in press). Outbreaks of the virus would likely act in concert to alter the spatial structure and population dynamics of this social species. In conjunction with direct mortality, these alterations in juvenile ecology have implications for future adult abundance and the overall sustainability of the Caribbean spiny lobster fishery.

Acknowledgements

We thank Dr. C. Li and K. Wheeler for help with the histology. Drs. R. Ratzlaff and R. Cooper critiqued the manuscript. This work was supported by NOAA Saltonstall-Kennedy Program Grant No. NA17FD2366 and NSF Biological Oceanography Program Grants # OCE-0136894 and OCE-0452805. We thank the numerous students and technicians that assisted in field and laboratory experiments, and staff at the Florida Marine Research Institute, Marathon Field Laboratory and Keys Marine Laboratory for facilities and logistical assistance. **[SS]**

References

Bachère, E., Gueguen, Y., Gonzalez, M., de Lorgeril, J., Garnier, J., Romestand, B., 2004. Insights into the anti-microbial defense of marine invertebrates: the penaeid shrimps and the oyster Crassostrea gigas. Immunol. Rev. 198, 149–168.

Behringer, D.C., Butler, M.J., 2006. Density-dependent population dynamics in juvenile Panulirus argus (Latrielle): the impact of artificial density enhancement. J. Exp. Mar. Biol. Ecol. 334, 84–95.

Behringer, D.C., Butler, M.J., Shields, J.D., 2006. Avoidance of disease in social lobsters. Nature 441, 421.

Braid, B.A., Moore, J.D., Robbins, T.T., Hedrick, R.P., Tjeerdema, R.S., Friedman, C.S., 2005. Health and survival of red abalone, Haliotis rufescens, under varying temperature, food supply, and exposure to the agent of withering syndrome. J. Invertebr. Pathol. 89, 219–231.

Brock, J.A., Lightner, D.V., 1990. Diseases caused by microorganisms. In: Kinne, O. (Ed.), Diseases of Marine Animals. Diseases of Crustacea, vol. III. Biologische Anstalt Helgoland, Hamburg, pp. 245–349.

Butler, M.J., Herrnkind, W.F., Hunt, J.H., 1997. Factors affecting the recruitment of juvenile Caribbean spiny lobsters dwelling in macroalgae. Bull. Mar. Sci. 61, 3–19.

Butler, M.J., Behringer, D.C., Shields, J.D., in press. Transmission of Panulirus argus virus 1 (PaV1) and its effect on the survival of juvenile Caribbean spiny lobster. Dis. Aquat. Org.

Curtis, L.A., 1987. Vertical distribution of an estuarine snail altered by a parasite. Science 235, 1509–1511.

Depledge, M.H., Bjerregaard, P., 1989. Haemolymph protein composition and copper levels in decapod crustaceans. Helgo Meeresunters 3, 207–223.

Dobson, A.P., 1988. The population biology of parasite-induced changes in host behavior. Q. Rev. Biol. 63, 139–165.

Dolan III, T.W., Butler, M.J., 2006. Modeling ontological changes in the social behavior of juvenile Caribbean spiny lobster, Panulirus argus. J. Crust. Biol. 26, 565–578.

Duffy, M.A., Hall, S.R., Tessier, A.J., Huebner, M., 2005. Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? Limnol. Oceanogr. 50, 412–420.

Eggleston, D.B., Lipcius, R.N., 1992. Shelter selection by spiny lobster under variable predation risk, social conditions, and shelter size. Ecology 73, 992–1011.

Goulson, D., 1997. Wipfelkrankheit: modification of host behaviour during baculoviral infection. Oecologia 109, 219–228.

Hamilton, W.D., Zuk, M., 1982. Heritable true fitness and bright birds: a role for parasites? Science 218, 384–387.

Hart, B.L., 1988. Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12, 123–137.

Hart, B.L., 1990. Behavioral adaptations to pathogens and parasites: five strategies. Neurosci. Biobehav. Rev. 14, 273–294.

Herrnkind, W.F., Butler, M.J., Hunt, J.H., Childress, M., 1997. The role of physical refugia: implications from a mass sponge die-off in a lobster nursery in Florida. Mar. Freshw. Res. 48, 759–769.

Holmes, J.C., Bethel, W.M., 1972. Modification of intermediate host behavior by parasites. In: Canning, E.U., Wright, C.A. (Eds.), Behavioural aspects of parasite transmission. Academic Press, London, pp. 123–149.

Horton, D.R., Moore, J., 1993. Behavioral effects of parasites and pathogens in insect hosts. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), Parasites and pathogens of insects, vol. 1. Academic Press, New York, pp. 107–124.

Johnson, P.T.J., Preu, E.R., Sutherland, D.R., Romansic, J.M., Han, B., Blaustein, A.R., 2006. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. Ecology 87, 2227–2235.

Lefcort, H., Eiger, S.M., 1993. Antipredatory behavior of feverfish tadpoles: implications for pathogen transmission. Behavior 126, 13–27.

Lefcort, H., Blaustein, A.R., 1995. Disease, predator avoidance, and vulnerability to predation in tadpoles. Oikos 74, 469–474.

Li, C., Shields, J.D., Ratzlaff, R.E., Butler IV, M.J., 2008. Pathology and hematology of the Caribbean spiny lobster experimentally infected with Panulirus argus virus 1 (PaV1). Virus Res. 132, 104–113.

Loehle, C., 1995. Social barriers to pathogen transmission in wild animal populations. Ecology 76, 326–335.

Lyle, W.G., MacDonald, C.D., 1983. Molt stage determination in the Hawaiian spiny lobster Panulirus marginatus. J. Crust. Biol. 3, 208–216.

MacDonald, D.W., 1980. Rabies and Wildlife: a biologist's perspective. Oxford University Press, Oxford.

Mintz, J.D., Lipcius, R.N., Eggleston, D.B., Seebo, M.S., 1994. Survival of juvenile Caribbean spiny lobster: effects of shelter size, geographic location, and conspecific abundance. Mar. Ecol. Prog. Ser. 112, 255–266.

Montgomery-Fullerton, M.M., Cooper, R.A., Kauffman, K., Shields, J.D., Ratzlaff, R.E., 2007. Detection of Panulirus argus virus 1 by PCR in Caribbean spiny lobsters. Dis. Aquat. Org. 76, 1–6.

Moore, J., 1983. Responses of an avian predator and its isopod prey to an acanthocephalan parasite. Ecology 64, 1000–1015.

Musgrove, R.J.B., 2001. The use of hemolymph chemistry in condition assessment of the southern rock lobster (Jasus edwardsii). J. Shell. Res. 19, 677.

Oliver, M.D., MacDairmid, A.B., 2001. Blood refractive index and ratio of weight to carapace length as indices of nutritional condition in juvenile rock lobsters (Jasus edwardsii). Mar. Freshw. Res. 52, 1395–1400.

Packer, C., Holt, R.D., Hudson, P.J., Lafferty, K.D., Dobson, A.P., 2003. Keeping the herds healthy and alert: implications of predator control for infectious disease. Ecol. Lett. 6, 797–802.

Poulin, R., 1995. "Adaptive" changes in the behavior of parasitized animals: a critical review. Int. J. Parasitol. 25, 1371–1383.

Shields, J.D., Scanlon, C., Volety, A., 2003. Aspects of the pathophysiology of blue crabs, Callinectes sapidus, infected with the parasitic dinoflagellate Hematodinium perezi. Bull. Mar. Sci. 72, 519–535.

Shields, J.D., Behringer, D.C., 2004. A new pathogenic virus in the Caribbean spiny lobster Panulirus argus from the Florida Keys. Dis. Aquat. Org. 59, 109–118.

Shields, J.D., Stephens, F.J., Jones, J.B., 2006. Chapter 5: Pathogens, parasites and other symbionts. In: Phillips, B.F. (Ed.), Lobsters: biology, management, aquaculture and fisheries. Blackwell Scientific Press, Oxford, pp. 146–204.

Smith, D.M., Dall, W., 1982. Blood protein, blood volume and extracellular space relationships in two Penaeus spp. (Decapoda: Crustacea). J. Exp. Biol. Ecol. 63, 1–15.

Vyas, A., Kim, S., Giacomini, N., Boothroyd, J.C., Sapolsky, R.M., 2007. Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. Proc. Natl. Acad. Sci. USA 104, 6442–6447.

Zieman, J.C., Fourqurean, J.W., Iverson, R.L., 1989. Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. Bull. Mar. Sci. 44, 292–311.