A Review of the Lethal Spiny Lobster Virus PaV1 – Ten Years After Its Discovery

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ABSTRACT

In 1999, we discovered that juvenile Caribbean spiny lobsters (*Panulirus argus*) in the Florida Keys were infected with PaV1 (*Panulirus argus* virus 1), the first naturally occurring pathogenic virus reported from lobsters. The virus profoundly affects their biology and ecology. PaV1 is probably wide-spread in the Caribbean with confirmed infections from the United States (Florida), St. Croix, Mexico, and Belize; and anecdotal reports from the Bahamas and Cuba. Mean prevalence in the Florida Keys has been stable since 1999 (5 - 8%), but has risen from 2.7% to 10.9% in Mexico (Puerto Morelos), the only other country where it has been studied extensively. The disease is most prevalent (> 15%) in the smallest juveniles lobsters (< 20 mm carapace length) and declines in prevalence among larger juveniles and adults. Although adults do not present the characteristic signs of this disease, they can harbor the virus with PCR-confirmed infections of adult, fishery-caught lobsters of 11 and 50% in Florida and Belize, respectively. The virus is lethal; infected lobsters die over one to several months with more rapid mortality for small juveniles. Infected lobsters become increasingly sedentary and cease feeding, often dying of metabolic exhaustion. Routes of viral transmission include ingestion, contact, and for early benthic juveniles, transmission through seawater over a few meters. Recent studies show that PaV1 is not viable in seawater for more than a few days, but larvae and postlarvae can be carriers over potentially long distances. Lobster ecology is dramatically altered during the course of infection. Prior to infectiousness, healthy lobsters avoid diseased lobsters, presumably reducing their risk of infection and resulting in infected juvenile lobsters dwelling alone rather than in groups. Avoidance results in increased shelter competition between healthy and diseased lobsters, with greater predation on the increasingly lethargic and solitary infected lobsters. Little is known about the prevalence or impact of PaV1 outside of Mexico and the United States, but the disease threatens fisheries throughout the pan-Caribbean range of *P. argus*. Marine diseases are emerging at an accelerated rate and the tools and knowledge that we develop through the study of diseases such as PaV1 will be invaluable in addressing future epizootics.

KEY WORDS: *Panulirus argus*, disease, epidemiology, ecology, behavior, prevalence, transmission

La Ecología y la Epidemiología de una Enfermedad Vírica en la Langosta con Púas del Caribe, *Panulirus argus*

En 1999 descubrimos un virus letal (PaV1) que infecta a la langosta espinosa del Caribe (*Panulirus argus*). Posteriores infecciones han sido confirmadas en la Florida, México, Belice y las Islas Vírgenes. Nosotros sospechoso PaV1 existe en otra parte en la gama de *P. argus,* pero estas áreas se quedan ser valorados. PaV1 es la primera enfermedad viral que se conoce afecta a la langosta, alterando el comportamiento y ecología de esta especie de manera fundamental. La prevalencia de esta infección es inversa al tamaño de las langostas, es decir que la mayoría de las infecciones se dan en las clases de menor tamaño. En la Florida, la prevalencia media del virus PaV1 durante las primeras etapas de las langostas juveniles en estado béntico es de aproximadamente el 20% aunque puede llegar a un 60% entre juveniles de mayor tamaño en áreas mas localizadas. La evidencia reciente sugiere que algunas langostas pueden abrigar PaV1 por períodos largos sin infecciones abiertas reveladoras. Hemos demostrado transmisión del virus a través de inyecciones de hemolinfa o ingestión de tejido provenientes de donantes infectados, contacto directo con las langostas infectadas, y a través del agua exclusivamente entre las langostas más pequeñas. Sin embargo, otros decapdos que co-ocupan guaridas con la langosta (cangrejo de piedra, *Menippi mercenaria*; cangrejo espinoso o centolla, *Mithrax spinosissimus*; langosta manchada, *P. guttatus*) no contraen el virus. Lo más notable de nuestros resultados es que las langostas sanas, normalmente sociales, pueden detectar y de hecho evitan el contacto con individuos infectados antes de contraer el virus. Este comportamiento, junto con una mayor depredación de individuos infectados puede romper la relación dependencia de densidad prevista de infección.

PALABRAS CLAVES: Enfermedad, virus, transmisión, predacion, letal

Écologie et Épidémiologie d'une Maladie Virale dans le Homard Épineux des Caraïbes, *Panulirus argus*

En 2000, nous avons découvert un virus mortel (PaV1) qui infecte les langoustes des Caraïbes (*Panulirus argus*). Les infections ont été confirmées en Floride, au Mexique, à Belize et des Îles Vierges. Nous suspectons que le PaV1 existe ailleurs dans la gamme de *P. argus*, mais ces secteurs restent à être évalués. PaV1 est la première maladie virale connue des homards et il change le comportement et l'écologie de cette espèce de façons fondamentales. La fréquence d'infection est inversement proportionnelle à la taille du homard, avec la plupart des infections se produisant parmi les classes de taille les plus petites. En Floride, la fréquence moyenne de PaV1 chez les premiers homards juvéniles benthiques est approximativement de 20 % et peut s'approcher de 60 % parmi de plus grands juvéniles dans quelques secteurs localisés. Une évidence récente suggère que quelques homards puissent héberger le PaV1 durant de longues périodes sans développer des infections manifestes. Nous avons démontré que la transmission par injection d'hémolymphe de donneurs infectés, ingestion de tissu infecté, contacts avec des homards infectés et - parmi les homards les plus petits - par l'eau. Les décapodes qui co-occupent des repaires avec le homard (crabe pierre, *Menippi mercenaria*; channel clinging crab, *Mithrax spinosissimus*; spotted crab, *P. guttatus*) ne contractent pas le virus. Le plus remarquable est que le homard en bonne santé, qui sont normalement sociable, détecte et évite leurs congénères malades avant de devenir infectieux. Ce comportement, avec une plus grande prédation entre les langoustes infectées, peut briser la densité de la prévalence attendue

infection.

MOTS CLÉS: Maladie, virus, transmission, prédation, mortel

INTRODUCTION

Until our discovery in 1999 of PaV1 (*Panulirus argus* virus 1) infections in juvenile Caribbean spiny lobster (*Panulirus argus*) in Florida, naturally occurring viral infections were unknown in lobsters (Behringer 2003, Shields and Behringer 2004). Spiny lobsters are afflicted by pathogens other than viruses (Shields *et al.* 2004) and decapod crustaceans (i.e., lobsters, crabs, and shrimp) as a whole, are subject to microbial and parasitic diseases (Kinne 1990) that sometimes cause epizootics with enormous impacts on fisheries. The extent of PaV1 prevalence and distribution throughout the pan-Caribbean distribution of *Panulirus argus* is unknown, but positive reports are mounting. Caribbean spiny lobsters are the target of the most economically valuable fishery in the Caribbean, where populations are considered fully- or over -exploited (FAO 2006). The discovery of PaV1 has put many fishery managers on high alert and some countries are taking steps to determine the extent of impact on their resource.

Since its discovery a decade ago, many advances have been made in understanding the pathology, epidemiology, ecology, and possible fishery implications of this novel disease. There has also been a suite of laboratory techniques developed to assess infection in field and laboratory. Here, we present the first comprehensive overview of the current state of knowledge regarding this lethal pathogen.

Detection and Pathology

Detection — Symptoms of juvenile lobsters heavily infected by PaV1 include lethargy, chalky-white hemolymph (Figure 1), and sometimes a discolored carapace that is heavily fouled by epibionts (Shields and Behringer 2004). Adult lobsters infected with the virus present no obvious visual signs of infection by PaV1, nor do juveniles with light or early-stage infections. Thus, more precise methods for screening are necessary and have been developed. Histological assessment of lobster tissues for pathology is a sensitive assay, albeit a destructive technique (Shields and Behringer 2004). In 2006, a molecular polymerase chain reaction (PCR) assay was developed by Montgomery-Fullerton *et al*. (2007) to non-destructively detect infection, and was reported sensitive to 1.2 fg of purified viral DNA. The technique was later modified and used to confirm PaV1 in *P. argus* from Puerto Morelos, Mexico (Huchin-Mian *et al*. 2008). The use of this PCR assay along with histological examination of tissues has allowed more thorough and accurate assessments of PaV1 in wild stocks and laboratory experiments.

Other laboratory techniques have also been developed to observe and quantify PaV1 infection. Li *et al.* (2006) developed a sensitive and specific florescent *in situ* hybridization (FISH) assay to detect PaV1 in the tissues of infected lobsters. Li and Shields (2007) also developed a culture method to allow *in vitro* studies of PaV1, and they successfully maintained hyalinocytes and semigranulocytes in culture and infect them *in vitro* with the PaV1 virus.

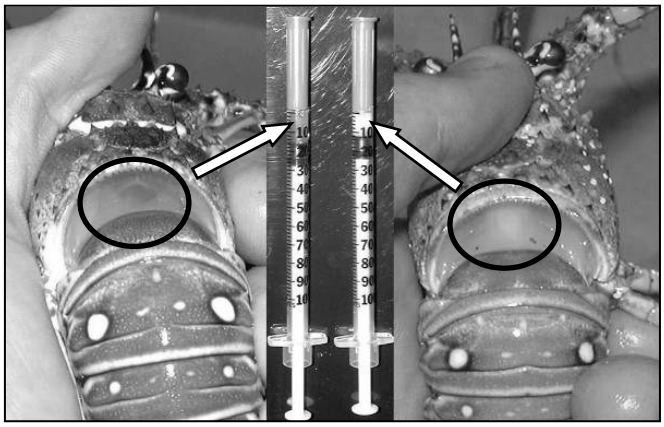

Figure 1. Comparison of hemolymph color between healthy (left; clear hemolymph) and PaV1-infected (right; chalky-white hemolymph) lobsters. (Photographs by Don Behringer)

Pathology — PaV1 was initially observed in juvenile lobster tissues using light microscopy and transmission electron microscopy (TEM) (Figure 2). Infection initially targets the fixed phagocytes of the hepatopancreas (i.e., digestive gland), and soon after infects certain circulating hemocytes (blood cells) (Li *et al*. 2008); specifically, hyalinocytes and semi-granulocytes but not granulocytes (Shields and Behringer 2004). Virions have also been observed in the spongy connective tissues of heavily infected individuals (Shields and Behringer 2004, Li *et al*. 2008). Transmission electron microscopy revealed infected cells with hypertrophied nuclei containing emarginated chromatin and icosahedral nucleocapsids approximately 182 ± 9 nm (SD) (Shields and Behringer 2004). Thus, virion assembly appears to occur entirely within the nucleus. Heavily infected lobsters also have a notable lack of reserve inclusions, indicative of a lack of glycogen reserves, supporting the hypothesis that mortality results from metabolic exhaustion (Shields and Behringer 2004). Indeed visibly infected lobsters have a significantly lower mean hemolymph refractive index, indicative of poor nutritional condition resulting from cessation of feeding (Behringer *et al*. 2008, Briones-Fourzan *et al*. 2009). However, poor nutritional condition does not appear to increase their initial risk of contracting PaV1 (Behringer *et al*. 2008). Susceptibility to infection and the progression of infection are partially dependent on lobster size (Butler *et*

al. 2008), with the smallest lobsters most susceptible and dying the fastest.

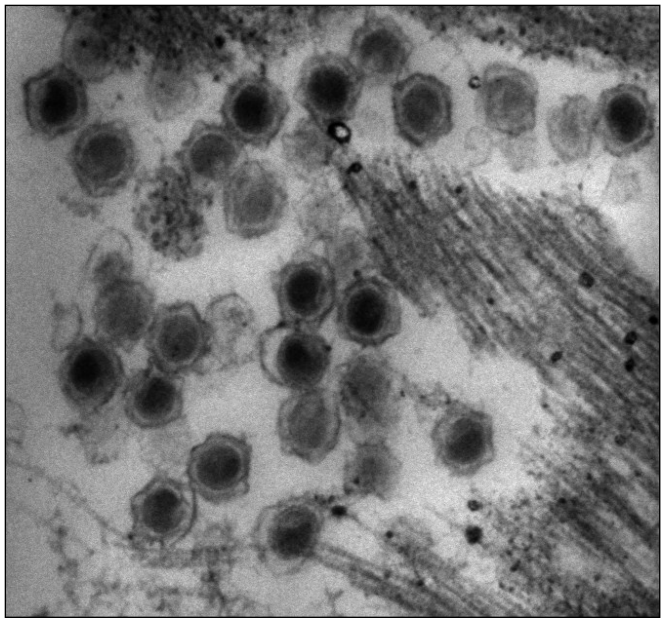

Figure 2. TEM image showing PaV1 virions loose within the hemolymph and among the abdominal muscle fibers of a heavily infected juvenile lobster. (Photograph by Don Behringer)

Epidemiology

Juvenile Lobsters — We have continued a long-term annual monitoring survey of juvenile lobsters at 12 sites in the middle and lower Florida Keys (USA) since 1999. This survey, begun prior to the advent of more precise detection techniques, was initiated as a visual disease survey and has been maintained as such. It underestimates true prevalence but is useful for observing temporal trends in prevalence. Mean prevalence of visible PaV1 infections in those surveys has remained constant, fluctuating between 5 - 8%. However, prevalence varies both spatially and temporally among the sites with some locales reaching > 50% infection in a given year. In 2002, we also performed a comprehensive survey of PaV1 prevalence in juvenile and sub-adult lobsters at 120 hard-bottom nursery sites throughout the Florida Keys from Key Largo to the Marquesas, west of Key West. Using histological screening, we found a mean PaV1 prevalence of 5% with no obvious spatial pattern in prevalence (Behringer, Butler, and Shields unpublished data). To date, the only other published information on PaV1 prevalence comes from the Yucatan coast of Mexico. Lozano-Alvarez *et al.* (2008) monitored the prevalence of PaV1 in the Mexican reef lagoon of Puerto Morelos and the oceanic atoll-reef of Chinchorro Bank using artificial casitas and natural shelters. Prevalence in the lagoon in 2001 was 2.7%, but increased to 7% in 2005 and to 10.9% in 2006, while the prevalence at Chinchorro Bank in 2006 was 7.4%.

PaV1 prevalence is highest (> 15%) among the smallest (< 20 mm CL) early benthic juveniles (EBJs) (Butler and Behringer unpublished data) and declines with lobster size (Figure 3). This pattern observed in Florida is similar to that found in Puerto Morelos and Chinchorro Bank (Lozano-Álvarez *et al*. 2008). The inverse relationship between PaV1 prevalence and lobster size may result from the combined effects of increasing immunological resistance with lobster size, decreasingly effective waterborne transmission with size (Butler *et al*. 2008), and the ability for healthy lobsters to detect infection in conspecifics (Behringer *et al.* 2006).

PaV1 has also been confirmed from wild lobster populations in St. Croix and Belize, with anecdotal reports from Cuba and the Bahamas (Butler *et al*. 2008, Huchin-Mian et al 2008, 2009) (Figure 4). It is also the suspected agent in mass mortalities of juvenile lobsters in aquaculture operations in Florida (Matthews and Maxwell 2007) and Belize (Staine and Dahlgren 2005). We suspect that it is widespread, possibly throughout the pan-Caribbean range of *P. argus*. Although laboratory studies suggest that the virus does not appear to remain viable as an infectious agent in the water for more than a few days (Li, Shields, Behringer and Butler, Unpublished data), recent findings indicate that postlarvae can harbor the virus and may contribute to its spread among regions (Butler, Behringer, Moss, Shields Unpublished data).

Adult Lobsters — Although PaV1 has its greatest impact on small juvenile lobsters, it also occurs in adults. In 2000, visual-based diver surveys of PaV1 prevalence in reef-dwelling adult lobsters throughout the Florida Keys

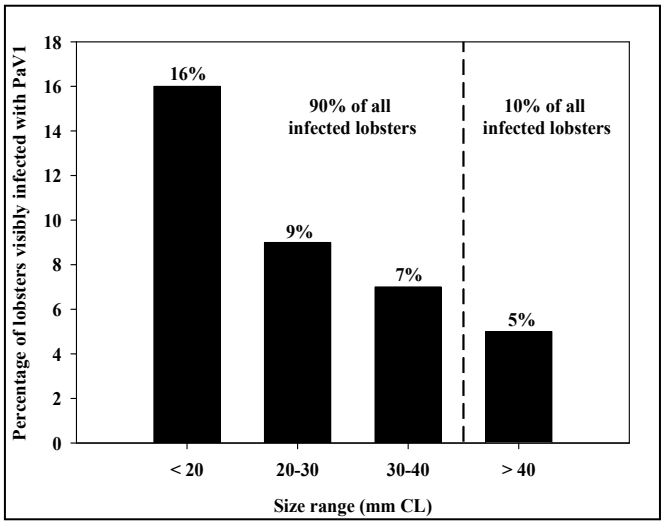

Figure 3. Map of the Caribbean showing the areas where PaV1 infection has been reported. Black X's represent areas where PaV1 has been confirmed to date. White X's represent areas where PaV1 has been anecdotally reported. The background map is courtesy of the University of Alabama Cartographic Research Laboratory.

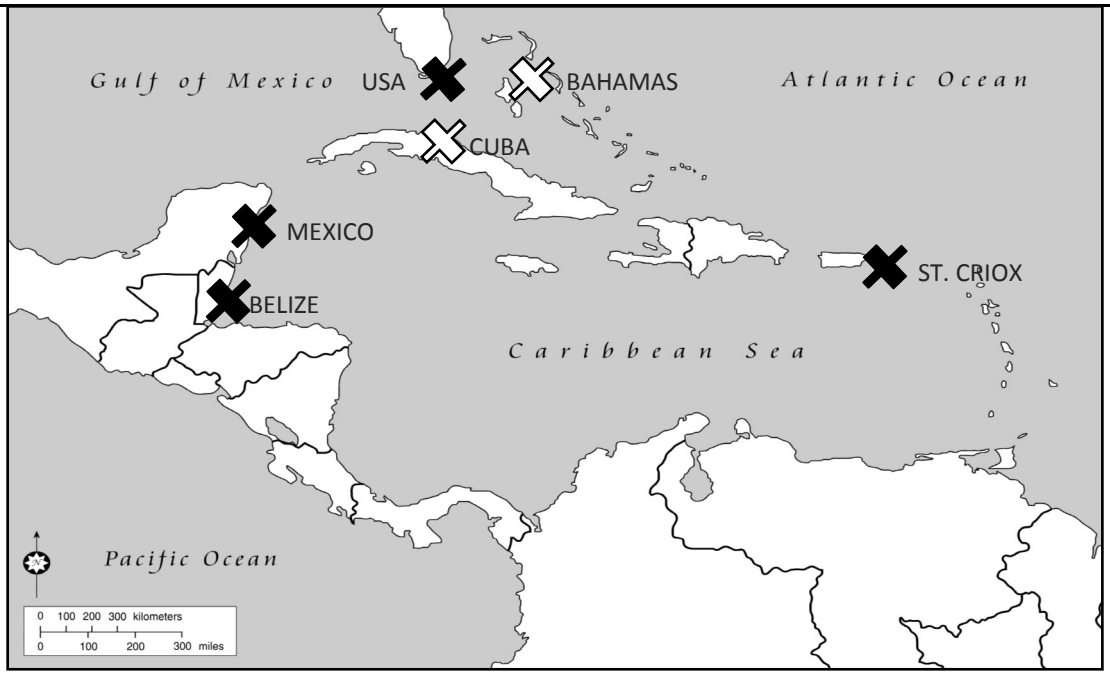

Figure 4. Prevalence of infection in juvenile Caribbean spiny lobster by size class for the combined data from field surveys at sites in the middle and lower Florida Keys in 2000-2001.

suggested a prevalence of < 1% (n = 4 of 1531; Shields and Behringer 2004). However, in 2008 – 2009 more accurate PCR-based screening of adult lobsters obtained from commercial fishery traps throughout the Florida Keys indicates that 11% of adults are infected with PaV1, although none displayed overt signs of infection. Huchin-Mian *et al*. (2009) also reported the discovery of PaV1 in the frozen tails of lobsters exported to Mexico from Belize. They found 50% (n = 11 of 22) of the sub-adult/adult tails to be PCR-positive for the disease.

Transmission

Transmission of PaV1 is feasible via several pathways, although not all are equally likely or efficient (Table 1). Transmission modes tested include inoculation, ingestion, contact, and waterborne (Butler *et al*. 2008). The latter two are the most likely modes of transmission in the wild, although clearly not as efficient as inoculation according to transmission coefficients calculated from the laboratory experiments (Butler *et al.* 2008). Waterborne transmission was only successful among EBJ and small juvenile lobsters (< 25 mm CL) over distances of 2 m or less, which may partially explain the high prevalence of PaV1 infection among these size lobsters in the wild. Ingestion of infected tissue remains a possible mode of transmission in the wild, but cannibalism is not observed or reported as common outside of laboratory settings, and a PaV1 reservoir species has not been identified. Transmission of PaV1 via inoculation to other potential host decapods (channel crab, *Mithrax spinosissimus*; stone crab, *Menippe mercenaria*; spotted lobster, *Panulirus guttatus*) that co-occur with *P. argus* has been tested but histological examination of their tissues 80 days post-inoculation revealed no active infections (Butler *et al*. 2008).

Nutritional condition of the lobster host appears to have little effect on their susceptibility to PaV1 infection (Behringer *et al*. 2008), nor does exposure to different seawater salinities (Behringer and Butler Unpublished data). No seasonal patterns of prevalence are apparent in the wild (Behringer 2003), but laboratory studies indicate that high seawater temperatures increase the susceptibility of EBJ lobsters to PaV1 infection, but not larger juveniles (Behringer and Butler Unpublished data).

Ecology and Behavior

Avoidance of Disease — In the wild, infected lobsters are virtually always alone (94% solitary) whereas healthy lobsters often co-occupy dens (46% solitary) (Behringer *et al.* 2006). Healthy individuals avoid diseased lobsters, while infected lobsters remain attracted to both healthy and diseased lobsters. Moreover, the onset of this avoidance behavior occurs prior to the time when diseased individuals become infectious. This appears to be effective at reducing contact transmission in this normally social species, because prevalence is independent of lobster density at the small spatial scales over which they interact (i.e., tens of meters) in nature (Behringer 2003, Lozano *et al*. 2008). However, the size and dimensions of the shelter may affect the frequency of shelter cohabitation as healthy lobsters co-occurred more frequently with diseased lobsters

Table 1. Results of PaV1 laboratory transmission trials (Butler *et al*. 2008). Note not all PaV1 exposed lobsters survived to the end of the trials.

Mode	Size range (mm CL)	Sample size	Trial duration (d)	Percent transmission	Transmission coefficient
Inoculation	30 – 55	21	80	95%	0.135
Ingestion	19 - 34	28	80	42%	0.005
Contact	20 – 30	15	80	63%	0.115
	30 – 40	15	80	33%	0.044
	40 – 50	15	80	11%	0.013
Waterborne	22 – 37	21	120	10%	0.026
	5 – 16	43	120	52%	0.004

in casitas (21.7% - 29.4%) than in smaller natural shelters (3.5%) (Lozano-Álvarez *et al*. 2008). Computer simulations using a spatially-explicit individual-based lobster recruitment model (Butler 2003, Butler *et al*. 2005, Dolan and Butler 2006) altered for modeling benthic disease dynamics in the Florida Keys (Dolan and Butler Unpublished data) also indicate that the avoidance of infected lobsters by healthy lobsters is very effective in dampening PaV1 prevalence in the population.

Movement and Predation — Heavily infected lobsters appear lethargic in the wild, and this tendency was demonstrated in a laboratory movement assay (Behringer *et al*. 2008). As infection progressed, PaV1 infected juvenile lobsters moved less, ultimately becoming sedentary. However, lobsters in the early stages of infection moved at rates similar to healthy lobsters, highlighting their potential to spread the disease throughout the population. Although visibly infected lobsters were largely sedentary in the laboratory, in 5-day mark-recapture experiments in the wild they were recaptured significantly less often than healthy lobsters, indicating that they were either emigrating more (unlikely given disease effects on movement) or suffering greater mortality (Behringer *et al*. 2008). Recent tethering experiments comparing the relative predation susceptibility between similar-sized healthy and infected lobsters showed greater predation inflicted upon disease lobsters regardless of the presence of shelter (Behringer and Butler In review).

Shelter Competition — The avoidance of shelters containing diseased lobsters has implications for healthy lobsters attempting to find shelter from predation, especially when shelter is limited (Behringer and Butler In review). The latter may occur in locations where structure for juveniles is naturally sparse, or when shelter (e.g., large sponges) is eliminated by a catastrophic event such as a harmful algal bloom or a disease outbreak (Butler *et al*. 1995). Shelter competition trials performed in shelter-limited mesocosms revealed that neither healthy nor diseased lobsters were dominant competitors for shelter, but the presence of a diseased lobster reduced cohabitation and thus increased the chance that one or both were excluded from shelter (Behringer and Butler In review). Shelter exclusion has more dire consequences for diseased lobsters, which suffer higher rates of predation.

Fishery

The Caribbean spiny lobster is the target of the most economically valuable fishery in the Caribbean (FAO 2006). However, fishery landings in Florida plummeted 30% in the 2000 – 2001 fishing season and have remained below historic levels, with the lowest landings ever reported from 2005 – 2006 (Ehrhardt *et al*. In press). Many factors affect fishery recruitment including loss of habitat (juvenile nursery or adult reefs), changes in spawning stocks and larval supply, changes in water quality, or catastrophic events (e.g., hurricanes and harmful algal blooms). Thus, pinpointing the cause of fishery declines is difficult. However, coincident with this downturn in fishery landings was the ominous discovery in 1999 of PaV1 (Shields and Behringer 2004) and the estimated loss of juveniles to this disease in Florida are similar in magnitude to the downturn in the fishery. We have no means of determining whether PaV1 truly emerged as a significant source of disease and mortality a decade ago, just prior to the downturn in the fishery, but the coincidence is ominous.

CONCLUSIONS

In the ten years since PaV1 was discovered, we have come a long way in understanding the nature of this pathogen and its effect on spiny lobsters. However, much remains to be done around the Caribbean to determine its true distribution and its impact on lobster populations and the marine communities and fisheries so dependent on this ecologically and economically important species. Although its prevalence in the Florida has remained relatively stable since its discovery, its prevalence in Mexico has increased sharply since 2001. It is unknown whether the latter pattern is a harbinger for other regions in the Caribbean, because so little is known of its impact or prevalence outside of Florida and Mexico. Marine diseases in general appear to be emerging at an accelerated rate (Harvell *et al*. 1999, 2002, 2004), so the tools and knowledge that we develop through study of diseases such

as PaV1 will be invaluable in addressing future epizootics.

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