



REVIEW

Review of *Panulirus argus* virus 1 — a decade after its discovery

Donald C. Behringer^{1,*}, Mark J. Butler IV², Jeffrey D. Shields³, Jessica Moss³

¹School of Forest Resources and Conservation and Emerging Pathogens Institute, University of Florida, Gainesville, Florida 32611, USA

²Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529, USA

³Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA

ABSTRACT: In 2000, a pathogenic virus was discovered in juvenile Caribbean spiny lobsters *Panulirus argus* from the Florida Keys, USA. *Panulirus argus* virus 1 (PaV1) is the first naturally occurring pathogenic virus reported from lobsters, and it profoundly affects their ecology and physiology. PaV1 is widespread in the Caribbean with infections reported in Florida (USA), St. Croix, St. Kitts, Yucatan (Mexico), Belize, and Cuba. It is most prevalent and nearly always lethal in the smallest juvenile lobsters, but this declines with increasing lobster size; adults harbor the virus, but do not present the characteristic signs of the disease. No other PaV1 hosts are known. The prevalence of PaV1 in juvenile lobsters from the Florida Keys has been stable since 1999, but has risen to nearly 11% in the eastern Yucatan since 2001. Heavily infected lobsters become sedentary, cease feeding, and die of metabolic exhaustion. Experimental routes of viral transmission include ingestion, contact, and for newly settled juveniles, free virus particles in seawater. Prior to infectiousness, healthy lobsters tend to avoid diseased lobsters and so infected juvenile lobsters mostly dwell alone, which appears to reduce disease transmission. However, avoidance of diseased individuals may result in increased shelter competition between healthy and diseased lobsters, and greater predation on infected lobsters. Little is known about PaV1 outside of Mexico and the USA, but it poses a potential threat to *P. argus* fisheries throughout the Caribbean.

KEY WORDS: *Panulirus argus* · Disease · Epidemiology · Ecology · Behavior · Prevalence · Transmission

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Until the discovery of *Panulirus argus* virus 1 (PaV1) (Shields & Behringer 2004), naturally occurring viral infections were unknown in lobsters. Other than PaV1, spiny lobsters are afflicted by non-viral pathogens (Shields et al. 2006, Shields 2011), and like other decapod crustaceans (i.e. lobsters, crabs, and shrimp) that are subject to a variety of microbial and parasitic diseases (Brock et al. 1990, Shields et al. 2006, Shields & Overstreet 2007), they sometimes cause epizootics with potential impacts on fisheries. The prevalence of PaV1 throughout the Caribbean range of *Panulirus argus*

is unknown, but reports of infections are mounting (Huchin-Mian et al. 2009, Cruz-Quintana et al. 2011). 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). Hence, the discovery of PaV1 is of concern and several countries are now taking steps to determine impacts of the virus on this valuable resource.

Since the initial description of PaV1 much has been done to understand its pathology, epidemiology, ecology, and possible fishery implications. A suite of techniques to assess and study PaV1 infection have also been developed. We review the current knowledge of

*Email: behringer@ufl.edu

PaV1; much has been learned about it, but there are many gaps that remain to be filled.

DETECTION AND PATHOLOGY

Detection

PaV1 pathology and virus particles were initially observed in tissues of lethargic juvenile *Panulirus argus* using light microscopy (Fig. 1) and transmission electron microscopy (TEM) (Fig. 2), respectively. TEM revealed icosahedral nucleocapsids $\sim 182 \pm 9$ nm (\pm SD) in infected cells with hypertrophied nuclei containing emarginated chromatin (Shields & Behringer 2004). Virions assemble in the nucleus and large aggregations of virions can be found free in the hemolymph. This double-stranded DNA virus currently remains unclassified, but it shares characteristics with both the *Iridoviridae* and the *Herpesviridae*. Gross signs of juvenile lobsters heavily infected by PaV1 include lethargy, chalky-white hemolymph (Fig. 3), and sometimes a discolored, heavily fouled carapace (Shields & Behringer 2004). Adult lobsters infected with the virus, along with juveniles with light infections, present no obvious gross signs. Histological detection of pathology is sensitive but destructive (Shields & Behringer 2004). In 2006, a molecular PCR assay was developed with a reported sensitivity to 1.2 fg of purified viral DNA (Montgomery-Fullerton et al. 2007). The PCR was later modified (the primer annealing temperature was increased from 51 to 63°C) and used to confirm PaV1 infection in *P. argus* from Puerto Morelos, Mexico (Huchin-Mian et al. 2008). The PCR has since been optimized to improve sensitivity to 0.05 fg of viral DNA (J. Moss et al. unpubl. data). A sensitive and specific

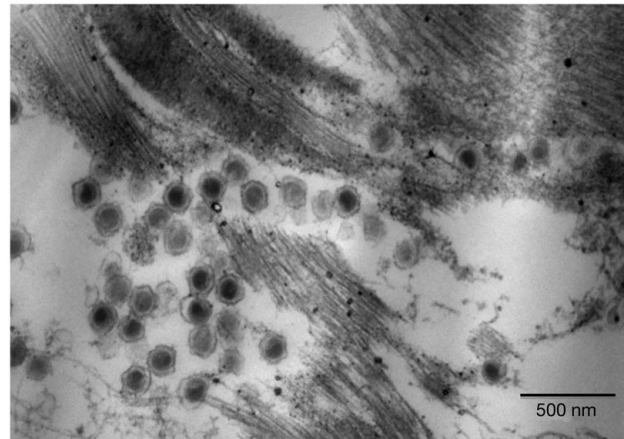


Fig. 2. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Transmission electron microscopy (TEM) image showing PaV1 virions loose within the hemolymph and among the abdominal muscle fibers of a heavily infected juvenile lobster

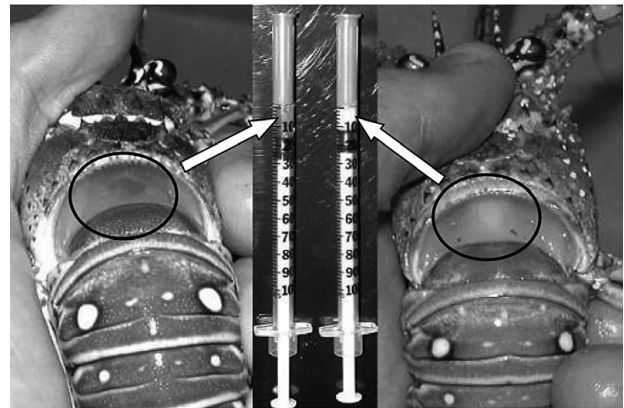


Fig. 3. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Comparison of hemolymph color between healthy (left syringe: clear hemolymph) and PaV1-infected (right syringe: chalky-white hemolymph) lobsters

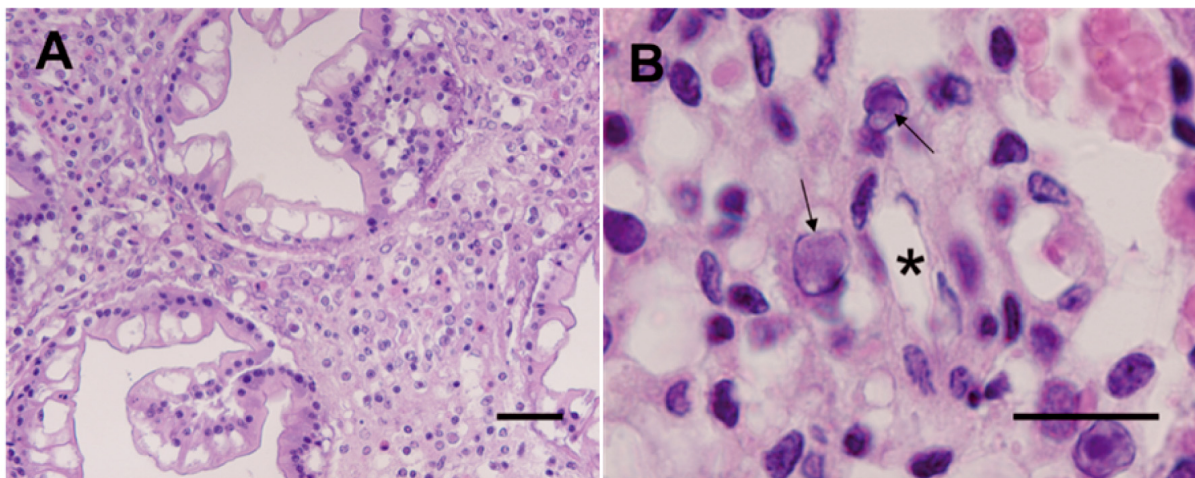


Fig. 1. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. (A) Atrophied hepatopancreas showing infiltration of hemocytes into the spongy connective tissues as a result of infection by PaV1. Scale bar = 50 μ m. (B) PaV1 infection in the fixed phagocytes (arrows) surrounding an arteriole in the hepatopancreas. *: lumen of the arteriole. Scale bar = 50 μ m

fluorescent *in situ* hybridization (FISH) assay has also been developed to visualize PaV1-infected lobster tissues (Li et al. 2006). The use of FISH confirmed that connective tissues of the hepatopancreas are the primary site of infection. Continuous cell cultures are not available for crustaceans. However, a primary cell culture method using semigranulocytes and hyalinocytes has been developed to quantify PaV1 in hemolymph samples (Li & Shields 2007). The quantal assay was based on virus-induced cytopathic effects in cell cultures infected in 10-fold serial dilutions of inocula. The assay could be used to calculate the tissue-culture infectious dose 50% (TCID₅₀) of the virus. These techniques now allow for more sensitive and accurate assessments of PaV1 in wild stocks and laboratory experiments.

Genetic information

Little genetic data currently exists for PaV1. The primers for the diagnostic PCR assay target a 500 bp fragment within a 892 bp fragment deposited in GenBank (accession number EF206313) (Montgomery-Fullerton et al. 2007). This DNA fragment appears to be an open reading frame with no other published viral homologs. The other sequenced piece of PaV1 DNA is a partial fragment of a DNA-directed polymerase (GenBank accession number DQ465025), to which the FISH probe was targeted (Li et al. 2006).

Pathology

PaV1 initially infects the fixed phagocytes of the hepatopancreas (i.e. digestive gland) and connective tissue cells surrounding the hepatopancreas (Li et al. 2008) (Fig. 1). Certain circulating hemocytes, specifically hyalinocytes and semi-granulocytes, are also infected (Shields & Behringer 2004). In heavily infected lobsters, virus-infected cells can be found in the spongy connective tissues surrounding most organs, with the hepatopancreas showing marked atrophy (Li et al. 2008). Heavily infected lobsters have a notable lack of reserve inclusions, indicative of a lack of glycogen reserves, supporting the hypothesis that mortality results from metabolic exhaustion (Shields & Behringer 2004). Several hemolymph constituents (glucose, phosphorus, triglycerides, and lipase A) were altered by infection, lending further support to this hypothesis (Li et al. 2008). Indeed heavily infected lobsters have a significantly lower mean hemolymph refractive index, indicative of poor nutritional condition resulting from cessation of feeding, and display a marked atrophy in the hepatopancreas (Behringer et al. 2008, Li et al.

2008, Briones-Fourzán et al. 2009). However, poor nutritional condition does not appear to increase their initial risk of contracting PaV1 (Behringer et al. 2008). The lethargy observed in heavy infections is likely an end stage of the disease due to poor nutritional condition and organ pathology.

EPIDEMIOLOGY

Juvenile lobsters

The prevalence of PaV1 in juvenile Caribbean spiny lobsters (ca. 20 to 55 mm carapace length, CL), as identified visually in long-term field surveys in the Florida Keys, has remained relatively constant, fluctuating between 2 and 8% (Fig. 4). However, prevalence varies both spatially and temporally among sites, with some localities reaching > 40% infection in a given year. In 2002, a more comprehensive survey was undertaken 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 histology to screen for PaV1, a mean prevalence of 5% was detected with no obvious spatial patterns (D. C. Behringer, M. J. Butler & J. D. Shields unpubl. data).

The prevalence of PaV1 is highest among the smallest (< 20 mm CL) early benthic juveniles (EBJs) (Butler et al. 2008) and declines with lobster size (Fig. 5). This pattern, observed in Florida, is similar to that observed in Puerto Morelos and Chinchorro Bank (Mexico)

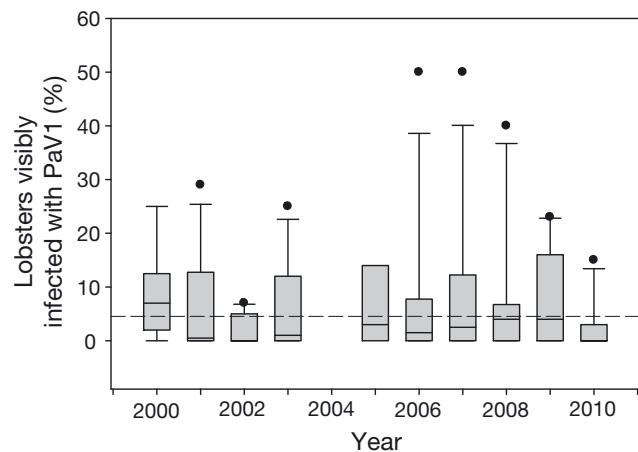


Fig. 4. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Box plots of visually detected PaV1 prevalence among all sizes of juvenile spiny lobsters from 12 sites in the middle and lower Florida Keys in 2000 to 2010. Individual box plots show the yearly geographic variability between the 12 sites. Dashed line represents the overall mean prevalence of 5%; black dots are outliers; whiskers represent 5th and 95th percentiles; solid line in box is median value for that size class

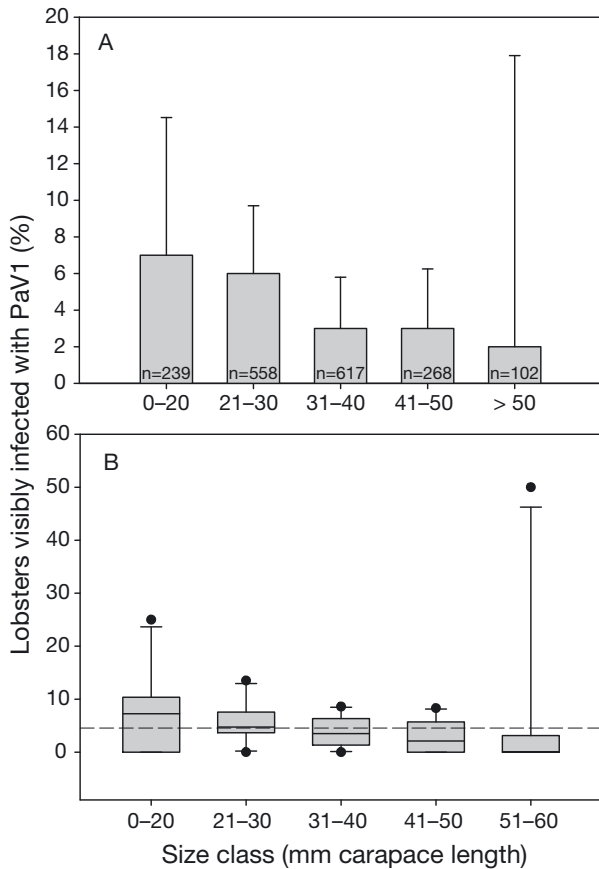


Fig. 5. *Panulirus argus* virus 1 (PaV1) infecting *Panulirus argus*. (A) Prevalence of late-stage visible PaV1 by size class for juvenile spiny lobsters from 12 sites in the middle and lower Florida Keys from 2000 to 2010. Error bars represent 1 SD and are based on the inter-annual variability. (B) Box plots of prevalence by size class. Dashed line represents the overall mean prevalence of 5%; black dots are outliers; whiskers represent 5th and 95th percentiles; solid line in box is median value for that size class

(Lozano-Álvarez et al. 2008). The inverse relationship between PaV1 prevalence and lobster size may result from the combined effects of decreasingly effective waterborne transmission with size (Butler et al. 2008)

and the ability for healthy lobsters to detect infected conspecifics (Behringer et al. 2006). However, recent PCR-based surveys of juveniles in Florida Bay in 2008 and 2010 show that surveys based on visual signs grossly underestimate the prevalence of PaV1 infection in juveniles (Table 1). Whether disease would develop in all of these PCR-positive individuals is under investigation but, regardless, PaV1 is more prevalent in juvenile lobsters in the Florida Keys than determined previously by visual or histological means (Shields & Behringer 2004).

PaV1 prevalence in lobsters occupying artificial and natural shelters has also been examined by visual means along the Yucatan coast of Mexico (Lozano-Álvarez et al. 2008). In 2001, PaV1 prevalence in the Mexican reef lagoon near Puerto Morelos was 2.7%, but increased to 7% in 2005 and to 10.9% in 2006; prevalence at the oceanic atoll-reef of Chinchorro Bank in 2006 was 7.4%. PaV1 has also been detected in wild lobster populations in St. Croix, St. Kitts, Belize, and Cuba, with anecdotal evidence of PaV1 infections

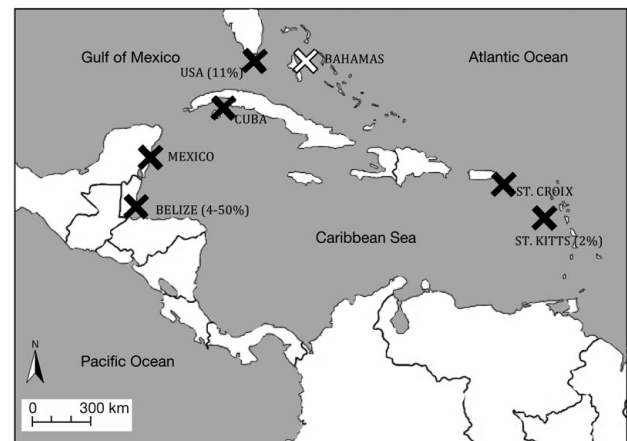


Fig. 6. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Map of the Caribbean showing the locations where PaV1 infection has been reported anecdotally (⊗) and confirmed (✱), along with the prevalence in adult lobsters (in parentheses). Background map is courtesy of the University of Alabama Cartographic Research Laboratory

Table 1. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Prevalence of PaV1 in the juvenile spiny lobster population from the Gulf of Mexico side of the middle Florida Keys from surveys in the summers of 2008 and 2010. CL: carapace length

Size class (mm CL)	June–August 2008				June–August 2010			
	Total lobsters	PCR+	Visibly diseased	PCR prevalence (%)	Total lobsters	PCR+	Visibly diseased	PCR prevalence (%)
0–20	28	11	0	39	13	8	0	62
21–30	52	20	0	38	23	7	0	30
31–40	63	16	0	25	70	27	1	39
41–50	43	8	0	19	34	15	0	44
>50	14	1	0	7	16	4	0	25
Total	200	56	0	28	156	61	1	39

being reported throughout the Caribbean (Butler et al. 2008, Huchin-Mian et al. 2008, 2009, Cruz-Quintana et al. 2011) (Fig. 6). Prevalence is also suspected to have caused mass mortalities of juvenile lobsters in aquaculture facilities in Florida (Matthews & Maxwell 2007) and Belize (Staine & Dahlgren 2005).

Adult lobsters

Although PaV1 has its greatest impact on small juvenile Caribbean spiny lobsters, it also occurs in adults. In 2001, diver-based visual surveys of adult lobsters throughout the Florida Keys indicated a prevalence of < 1% (n = 4 of 1531; Shields & Behringer 2004). However, in 2008 to 2009 more sensitive PCR-based screening of adult lobsters from commercial traps throughout the Florida Keys detected PaV1 in 11% of the lobsters (authors' unpubl. data). Similarly, PaV1 was detected by PCR in 50% (n = 11 of 22) of the sub-adult/adult frozen lobster tails imported in Mexico from Belize (Huchin-Mian et al. 2009). Prevalence of 4% was detected by PCR (n = 101) among tissues from adult lobsters (75–160 mm CL) recently collected in Belize. This discrepancy may have arisen due to cross-contamination of the exported tails prior to receipt in Mexico or from temporal and geographic variability in PaV1 prevalence within Belize.

TRANSMISSION

Transmission of PaV1 may occur via several pathways, although not all are equally likely or efficient (Table 2). Transmission routes tested include hemolymph inoculation, ingestion, contact, and waterborne routes (Butler et al. 2008). The latter 2 are the most likely natural modes of transmission (Butler et al. 2008). Waterborne transmission has only been demonstrated for 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 the smallest lobsters in the wild. Ingestion of infected tissue remains a possible mode of natural transmission, but cannibalism is probably uncommon outside of laboratory settings, and prey species that could serve as PaV1 reservoirs have not been identified. Transmission of PaV1 via infected hemolymph inoculation in other potential host decapods (channel crab *Mithrax spinosissimus*, stone crab *Menippe mercenaria*, spotted lobster *Panulirus guttatus*) that co-occur with *P. argus* have been unsuccessful based on histological examination of tissues 80 d post-inoculation, although tissues were not tested by PCR (Butler et al. 2008).

The nutritional condition of juvenile lobsters has no effect on their susceptibility to PaV1 infection (Behringer et al. 2008), nor does exposure to seawater differing in salinity (D. C. Behringer et al. unpubl. data). No seasonal patterns of prevalence are apparent in Florida lobster populations (Behringer 2003). However, laboratory studies indicate that high seawater temperatures increase the susceptibility of EBJ lobsters to PaV1 infection, but not larger juveniles (D. C. Behringer et al. unpubl. data). Susceptibility to infection and the progression of infection are also partially dependent on lobster size (Butler et al. 2008), with the smallest lobsters being most susceptible and dying the fastest.

ECOLOGY AND BEHAVIOR

Avoidance of disease

Panulirus argus are naturally gregarious and den together for protection under structure such as sponges, corals, and solution holes. Yet in the wild, infected lobsters occur alone (94% solitary) whereas healthy lobsters often co-occupy dens with other lobsters (46% solitary) (Behringer et al. 2006). Laboratory experi-

Table 2. *Panulirus argus* virus 1 (PaV1) infecting *P. argus*. Experimental transmission of PaV1 to juvenile spiny lobsters. All infections were detected using histological examination (data from Butler et al. 2008). CL: carapace length

Mode ^a	Lobster 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

^aNote: not all lobsters exposed to PaV1 survived to the end of the trials

ments revealed that healthy individuals detect and avoid diseased lobsters, whereas infected lobsters continue to be attracted to both healthy and diseased lobsters. The onset of avoidance behavior by healthy lobsters occurs just prior to the onset of infectiousness (Behringer et al. 2006), and computer simulations (Dolan 2010) and field studies (M. J. Butler et al. unpubl. data) indicate that this behavior is effective at reducing transmission in this normally gregarious species.

PaV1 prevalence in nature is independent of lobster density over the small spatial scales in which lobsters interact (i.e. 10s of meters) (Behringer 2003, Lozano-Álvarez et al. 2008). However, the size and dimensions of a shelter may affect the frequency of shelter cohabitation as healthy lobsters co-occur more frequently with diseased lobsters in large casitas (21.7 to 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 & Butler 2006) altered for modeling benthic disease dynamics in the Florida Keys have also indicated that the avoidance of infected lobsters by healthy lobsters is effective in dampening the prevalence of PaV1 in the population modeled (Dolan 2010).

Movement and predation

Heavily infected *Panulirus argus* appear lethargic in the wild, and this moribund behavior has been replicated 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 to new locations. In the wild, lobsters were recaptured significantly less often after 5 d than healthy lobsters, indicating that they were either emigrating in greater numbers or suffering greater mortality (Behringer et al. 2008). Recent tethering experiments comparing the relative predation susceptibility between similar-sized healthy and infected lobsters has confirmed that visibly diseased lobsters indeed experience higher predation than healthy lobsters regardless of the presence of shelter (Behringer & Butler 2010).

Shelter competition

The avoidance of diseased lobsters by healthy conspecifics has implications for lobster shelter acquisition and refuge from predation, especially when shelter is limited (Behringer & Butler 2010). The latter may occur

in locations where structure for juveniles is naturally sparse, or when shelter (e.g. large sponges) is eliminated by catastrophic events such as harmful algal blooms or disease outbreaks (Butler et al. 1995, Herrnkind et al. 1997). Shelter competition trials performed in shelter-limited mesocosms have revealed that neither healthy nor diseased lobsters are dominant competitors for shelter, but the presence of a diseased lobster reduces cohabitation and thus increases the chance that one lobster is excluded from shelter (Behringer & Butler 2010). Shelter exclusion has more dire consequences for diseased lobsters, which suffer higher rates of predation. However, cohabitation between diseased and healthy lobsters appears to occur more frequently in areas where shelter is scarce than in areas where shelter is abundant (Lozano-Álvarez et al. 2008). Perhaps some healthy lobsters make a trade-off between infection and predation risk in low shelter environments, as is thought to occur in the eastern Yucatan.

Fishery

The *Panulirus argus* fishery is the most economically valuable in the Caribbean (FAO 2006). However, in the 2000–2001 season fishery landings in Florida plummeted 30% from those reported the decade before and subsequently remained at these low levels, with the lowest landings ever reported occurring in 2005–2006; and similar declines have occurred elsewhere in the Caribbean (Ehrhardt et al. 2010). Many factors affect fishery recruitment including the loss of habitat for juveniles or adults, changes in spawning stocks and larval supply, changes in water quality, or environmental events that influence population dynamics (e.g. hurricanes, harmful algal blooms, and changes in oceanographic conditions or currents). Thus, pinpointing the cause of fishery declines is difficult, but some lobster fisheries have been severely impacted by other diseases (Wahle et al. 2009). Recent studies show that healthy lobsters can acquire PaV1 when confined in commercial fishing traps with infected lobsters for as little as 7 d, with even higher transmission when lobsters were held together for 14 d (D. C. Behringer et al. unpubl. data). Although the PaV1 disease was not described until 2004 (Shields & Behringer 2004), there are anecdotal reports from fishermen and scientists in Florida and elsewhere in the Caribbean of lobsters with characteristic PaV1 infections observed over 25 yr ago. Thus, it is unlikely that PaV1 is a newly emergent pathogen. However, the presence of a lethal, pathogenic virus infecting the Caribbean's most important fishery resource is of concern to resource managers in the region.

CONCLUSIONS

In the 10 yr since PaV1 was discovered, we now have a better understanding of the nature of this pathogen and how it affects Caribbean spiny lobsters *Panulirus argus*. However, much remains to be done in unstudied regions of the Caribbean to determine the prevalence and impact of PaV1 on lobster populations, fisheries, and fishing communities that are so dependent on this ecologically and economically important species. Although its prevalence in Florida has remained relatively stable since its discovery, its prevalence in the eastern Yucatan 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); therefore, the tools and knowledge on PaV1 gathered to date will be invaluable in addressing potential future epizootics.

Acknowledgements. The authors thank our many collaborators, students, and technicians that have assisted to produce this body of work. Funding for our research has been generously provided by the National Science Foundation (OCE-0452383, OCE-0452805, OCE-0136894) and the National Sea Grant College Program of the U.S. Department of Commerce's National Oceanic and Atmospheric Administration (NOAA) (Grant No. NA16RG-2195).

LITERATURE CITED

- Behringer DC (2003) The ecological ramifications of density and disease in the Caribbean spiny lobster *Panulirus argus*. PhD thesis, Old Dominion University, Norfolk, VA
- Behringer DC, Butler MJ IV (2010) Disease avoidance influences shelter use and predation in Caribbean spiny lobster. *Behav Ecol Sociobiol* 64:747–755
- Behringer DC, Butler MJ IV, Shields JD (2006) Avoidance of disease in social lobsters. *Nature* 441:421
- Behringer DC, Butler MJ IV, Shields JD (2008) Ecological and physiological effects of PaV1 infection on the Caribbean spiny lobster (*Panulirus argus* Latreille). *J Exp Mar Biol Ecol* 359:26–33
- Briones-Fourzán P, Baeza-Martínez K, Lozano-Álvarez E (2009) Nutritional indices of juvenile Caribbean spiny lobsters in a Mexican reef lagoon: Are changes over a 10-year span related to the emergence of *Panulirus argus* Virus 1 (PaV1)? *J Exp Mar Biol Ecol* 370:82–88
- Brock IA, Lightner DV, Meyers TR (1990) Diseases of Crustacea, Chap 3. In: Kinne O (ed) *Diseases of marine animals*, Vol III. Biologische Anstalt Helgoland, Hamburg, p 245–423
- Butler MJ IV (2003) Incorporating ecological process and environmental change into spiny lobster population models using a spatially-explicit, individual-based approach. *Fish Res* 65:63–79
- Butler MJ IV, Hunt JH, Herrnkind WF, Childress MJ and others (1995) Cascading disturbances in Florida Bay, USA: cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobster *Panulirus argus*. *Mar Ecol Prog Ser* 129:119–125
- Butler MJ IV, Dolan T, Hunt JH, Herrnkind WF, Rose K (2005) Recruitment in degraded marine habitats: a spatially-explicit, individual-based model for spiny lobster. *Ecol Appl* 15:902–918
- Butler MJ IV, Behringer DC, Shields JD (2008) Transmission of *Panulirus argus* virus 1 (PaV1) and its effect on the survival of juvenile Caribbean spiny lobster. *Dis Aquat Org* 79:173–182
- Cruz-Quintana Y, Silveira-Coffigny R, Rodríguez-Canul R, Vidal-Martínez VM (2011) First evidence of *Panulirus argus* Virus 1 (PaV1) in spiny lobster from Cuba and clinical estimation of its prevalence. *Dis Aquat Org* 93:141–147
- Dolan TW III (2010) The use of spatially-explicit, agent-based models to understand the effects of disease and environmental change on the recruitment of Caribbean spiny lobsters, *Panulirus argus*. PhD thesis, Old Dominion University, Norfolk, VA
- Dolan TW III, Butler MJ IV (2006) Modeling ontological changes in the social behavior of juvenile Caribbean spiny lobster, *Panulirus argus*. *J Crustac Biol* 26:565–578
- Ehrhardt NM, Puga R, Butler MJ IV (2010) Large ecosystem dynamics and fishery management concepts: the Caribbean spiny lobster, *Panulirus argus*, fisheries. In: Fanning L, Mahon R, McConney P (eds) *Towards marine ecosystem-based management in the wider Caribbean*. Amsterdam University Press, Amsterdam, p 157–175
- FAO (Food and Agriculture Organization) (2006) *The state of world fisheries and aquaculture*. FAO, Rome
- Harvell CD, Kim K, Burkholder JM, Colwell RR and others (1999) Emerging marine diseases—climate links and anthropogenic factors. *Science* 285:1505–1510
- Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. *Science* 296:2158–2162
- Harvell D, Aronson R, Baron N, Connell J and others (2004) The rising tide of ocean diseases: unsolved problems and research priorities. *Front Ecol Environ* 2:375–382
- Herrnkind WH, Butler MJ IV, Hunt JH, Childress MJ (1997) The role of physical refugia: implications from a mass sponge die-off in a lobster nursery. *Mar Freshw Res* 48:759–770
- Huchin-Mian JP, Rodríguez-Canul R, Arias-Bañuelos E, Simá-Álvarez R, Pérez-Vega JA, Briones-Fourzán P, Lozano-Álvarez E (2008) Presence of *Panulirus argus* Virus 1 (PaV1) in juvenile spiny lobsters *Panulirus argus* from the Caribbean coast of Mexico. *Dis Aquat Org* 79:153–156
- Huchin-Mian JP, Briones-Fourzán P, Simá-Álvarez R, Cruz-Quintana Y and others (2009) Detection of *Panulirus argus* Virus 1 (PaV1) in exported frozen tails of subadult-adult Caribbean spiny lobsters *Panulirus argus*. *Dis Aquat Org* 86:159–162
- Li C, Shields JD (2007) Primary cell culture of hemocytes from the spiny lobster and their susceptibility to *Panulirus argus* virus 1 (PaV1). *J Invertebr Pathol* 94:48–55
- Li C, Shields JD, Small HJ, Reece KS, Hartwig CL, Cooper R, Ratzlaff RE (2006) Detection of *Panulirus argus* Virus 1 (PaV1) in the Caribbean spiny lobster using fluorescence *in situ* hybridization (FISH). *Dis Aquat Org* 72:185–192
- Li C, Shields JD, Ratzlaff RE, Butler MJ IV (2008) Pathology and hematology of the Caribbean spiny lobster experimentally infected with *Panulirus argus* virus 1 (PaV1). *Virus Res* 132:104–113
- Lozano-Álvarez E, Briones-Fourzán P, Ramírez-Estévez A, Placencia-Sánchez D, Huchin-Mian JP, Rodríguez-Canul R

- (2008) Prevalence of *Panulirus argus* Virus 1 (PaV1) and habitation patterns of healthy and diseased Caribbean spiny lobsters in shelter-limited habitats. *Dis Aquat Org* 80:95–104
- Matthews TR, Maxwell KE (2007) Growth and mortality of captive Caribbean spiny lobsters, *Panulirus argus*, in Florida, USA. *Proc Gulf Caribb Fish Inst* 58:377–386
- Montgomery-Fullerton MM, Cooper RA, Kauffman K, Shields JD, Ratzlaff RE (2007) Detection of *Panulirus argus* Virus 1 in Caribbean spiny lobsters. *Dis Aquat Org* 76:1–6
- Shields JD (2011) Diseases of spiny lobsters: a review. *J Invertebr Pathol* 106:79–91
- Shields JD, Behringer DC (2004) A new pathogenic virus in the Caribbean spiny lobster *Panulirus argus* from the Florida Keys. *Dis Aquat Org* 59:109–118
- Shields JD, Overstreet RM (2007) Parasites, symbionts, and diseases. In: Kennedy V, Cronin LE (eds) *The blue crab *Callinectes sapidus**. University of Maryland Sea Grant College, College Park, MD, p 299–417
- Shields JD, Stephens FJ, Jones JB (2006) Pathogens, parasites and other symbionts, Chap 5. In: Phillips BF (ed) *Lobsters: biology, management, aquaculture and fisheries*. Blackwell Scientific, Oxford, p 146–204
- Staine F, Dahlgren C (2005) Experimental grow-out aquaculture of the Caribbean spiny lobster in Belize. *Book of Abstracts Proc Gulf Carib Fish Inst* 58:129
- Wahle RA, Gibson M, Fogarty M (2009) Distinguishing disease impacts from larval supply effects in a lobster fishery collapse. *Mar Ecol Prog Ser* 376:185–192

Editorial responsibility: Grant Stentiford, Weymouth, UK

*Submitted: October 7, 2010; Accepted: December 20, 2010
Proofs received from author(s): March 23, 2011*