PaV1 infection in the Florida spiny lobster (*Panulirus argus*) fishery and its effects on trap function and disease transmission

Donald C. Behringer, Mark J. Butler IV, Jessica Moss, and Jeffrey D. Shields

Abstract: The Caribbean spiny lobster (*Panulirus argus*) supports the most economically valuable fishery in the Caribbean. In Florida, USA, the majority of the catch is landed in traps "baited" with live, sublegal-sized lobsters that attract other lobsters due to their social nature. This species is also commonly infected by the pathogenic virus *Panulirus argus* Virus 1 (PaV1). Here we describe a polymerase chain reaction (PCR) based assessment of the prevalence of PaV1 in the lobster fishery from the Florida Keys. We tested the effect of PaV1-infected lobsters in traps on catch and on transmission to other trapped, uninfected lobsters. We found that 11% of the lobsters caught in commercial traps were positive for the virus by PCR, but none of these animals showed visible signs of disease. We also tested whether healthy lobsters avoid diseased lobsters in traps. Traps into which we introduced an infected lobster caught significantly fewer lobsters than traps containing an uninfected lobster. Moreover, uninfected lobsters confined in traps with infected lobsters acquired significantly more PaV1 infections than those confined with uninfected lobsters. This study demonstrates the indirect effects that pathogens can have on fisheries and the unintended consequences of certain fishery practices on the epidemiology of a marine pathogen.

Résumé : La pêche à la langouste des Caraïbes (*Panulirus argus*) représente une des pêches commerciales les plus importantes du point de vue économique dans les Caraïbes. En Floride, É.-U., la majorité des captures sont faites dans des casiers utilisant comme « appâts » des langoustes vivantes de taille inférieure à la taille légale qui attirent les autres langoustes à cause du caractère social de l'espèce. La langouste est aussi fréquemment infectée par le virus pathogène *Panulirus argus* virus 1 (PaV1). Nous déterminons la prévalence du PaV1 dans la population de langoustes dans les Keys de la Floride à l'aide de la réaction en chaîne par polymérase (PCR). Nous évaluons l'effet des langoustes infectées par PaV1 dans les casiers sur les captures et sur la transmission aux autres langoustes non infectées dans le casier. Dans les casiers commerciaux, 11 % des langoustes capturées affichent une réaction positive au virus à l'analyse PCR, mais aucun de ces animaux ne présente de signes apparents de la maladie. Nous testons aussi si les langoustes en santé évitent les langoustes infectées dans les casiers. Les casiers dans lesquels nous avons placé une langouste infectée capturent significativement moins de langoustes que les casiers contenant une langouste saine. De plus, les langoustes saines confinées dans des casiers contenant des langoustes infectées contractent significativement plus d'infections à PaV1 que celles enfermées avec des langoustes saines. Notre étude démontre les effets indirects que les pathogènes peuvent avoir sur les pêches commerciales et les conséquences imprévues de certaines pratiques de pêche sur l'épidémiologie d'un pathogène marin.

[Traduit par la Rédaction]

Introduction

Pathogens can have significant effects on host populations that support fisheries worldwide (Dobson and May 1987; Patterson 1996; Shields et al. 2005), among them valuable fisheries for decapod crustaceans (e.g., Shields et al. 2007; Shields and Overstreet 2007; Wahle et al. 2009). Several pathogens have impacted commercially important crustacean fisheries in recent decades. For example, parasitic dinoflagellates of the genus *Hematodinium* have caused considerable mortality impacting important crab and lobster fisheries (e.g., snow crabs, *Chionoecetes opilio* (Shields et al. 2005, 2007; Mullowney et al. 2011); velvet crab, *Necora puber*, (Wilhelm and Mialhe 1996); and Norway lobster, *Nephrops norvegicus* (Stentiford et al. 2001)). Epizootic shell disease has had a devastating effect on American lobster (*Homarus americanus*) in southern New England (USA) over the past decade (Castro and Angell 2000; Wahle et al. 2009), prompting the Atlantic States Marine Fisheries Commission – American Lobster Technical Committee to recom-

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mend a 5-year closure of the fishery. Spiny lobsters (Palinuridae) are also afflicted by a range of pathogens (Shields 2011*a*), but they rarely pose a risk to fisheries. An exception may be the PaV1 virus that infects the Caribbean spiny lobster (*Panulirus argus*).

Panulirus argus supports one of the most economically valuable fisheries in the Caribbean, with commercial landings in Florida, USA, valued at over $22M annually (Florida Fish and Wildlife Conservation Commission 2008), and whose value now approaches US$1B Caribbean-wide up from over US$800M five years ago (Food and Agriculture Organization of the United Nations (FAO) 2006). However, over the past decade, commercial landings of lobster in Florida and in several countries in the Caribbean have declined by ~30% and remain below historic levels (Southeast Data, Assessment, and Review 8 (SEDAR 8) 2005; Ehrhardt et al. 2010). The cause of this decline is unknown and difficult to discern because of a lack of time-series data chronicling changes in crucial environmental parameters, lobster population metrics, and fishery effort statistics. Stocks of *P. argus* are overexploited or nearly so in many areas of the Caribbean (Chávez 2009), and the loss of spawning stock may explain the general decline in many fisheries. However, the discovery of the pathogenic virus *Panulirus argus* Virus 1 (PaV1) infecting lobsters in the region is a major concern (Shields and Behringer 2004), and where prevalent, it is of undoubted consequence to lobster stocks and the fisheries they support.

Caribbean spiny lobsters are fished throughout their range using a number of different gear types, including traps, casitas (a flat surface of wood, metal, or cement braced approximately 15 cm off the seafloor), nets, and spears. In Florida, the primary commercial fishing gear is a wood- or plastic-slat trap, no larger than 0.9 m × 0.6 m. Currently, there are about 500 000 traps in the Florida fishery, down from 944 000 in 1992 (Vondruska 2010). There is growing concern over the potential introduction of pathogens from imported or spoiled bait used in fisheries (Hasson et al. 2006; Hervé-Claude et al. 2008), but little or no bait is used in the Florida fishery for *P. argus*. Instead, commercial trap fishermen use sublegal (legal = adult = 76 mm carapace length (CL)) lobsters as live "bait" in traps as a social attractant to legal-sized lobsters and are permitted to possess up to 50 sublegal lobsters ("shorts") and one per trap aboard each boat. Trap confinement of sublegal lobsters results in 29% mortality within four weeks of confinement in a trap, and an estimated 47% of all sublegal lobsters used in traps die during the course of the fishing season (Kennedy 1982; Hunt et al. 1986; Hunt 2000). Traps may also alter PaV1 transmission among spiny lobsters, and the disease may, in turn, alter trap efficiency.

PaV1-diseased lobsters disrupt the normal gregarious behavior of nondiseased lobsters (Behringer et al. 2006), so it is also possible that an infected lobster within a trap could diminish the efficiency of the trap by driving away future inhabitants. Stress induced from confinement and bait-induced malnutrition may also increase disease susceptibility (Tlusty et al. 2008). Transmission of PaV1 among lobsters may also be increased if a diseased lobster enters a trap containing healthy lobsters. The unintentional onboard transport of infected sublegal lobsters from one fishing location to another and potential spread of PaV1 is also a concern. Thus, the use of sublegal lobsters in fishing traps in Florida, combined with the unique changes in social behavior associated with PaV1 infection, creates a situation in which the pathogen and fishery may interact to influence disease dynamics in spiny lobsters.

Therefore, our objectives for this study were to determine (*i*) the prevalence and distribution of PaV1 in lobsters entering the fishery in the Florida Keys using a polymerase chain reaction (PCR) based diagnostic, (*ii*) how the presence of infected lobsters within lobster traps affects the trapping of other lobsters, and (*iii*) if trap confinement enhances PaV1 transmission among lobsters.

Materials and methods

PCR screening for PaV1

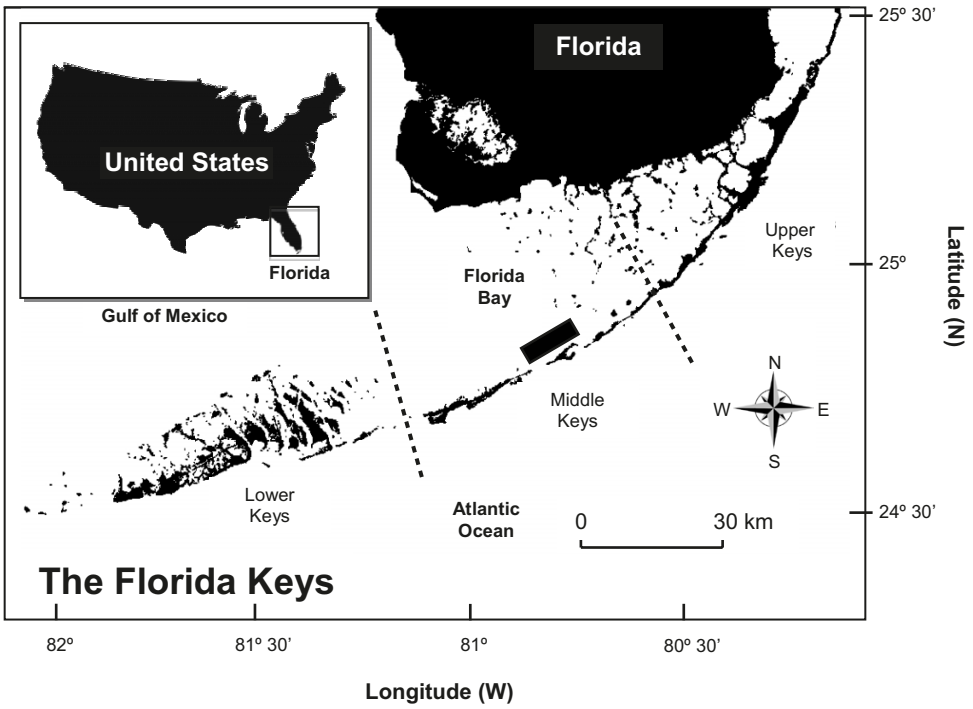
A diagnostic assay for PaV1 infection was used based on the primer set published by Montgomery-Fullerton et al. (2007) with modifications (Moss et al. 2012). In brief, DNA extractions were performed on 100–150 μL of lobster hemolymph using a Qiagen DNeasy (Valencia, California) blood and tissue kit, following the manufacturer's protocol. Lobster genomic DNA was eluted in 150 μL of extraction buffer and stored at 4 °C until assayed. The quality of genomic DNA was assessed by amplifying the small subunit ribosomal RNA (SSU) of the lobster using "universal" SSU primers modified from Medlin et al. (1988) (see Moss et al. 2006). The amplified target DNA fragment was approximately 1800 bp in length. PCR products from the SSU and PaV1 assays were loaded separately onto a 2% agarose gel (*w*/*v*), electrophoresed at 100V, stained with ethidium bromide, and examined under UV light. Images were recorded using the Alpha Innotech FlourChem (San Leandro, California) imaging system.

All lobsters used in experiments were prescreened for PaV1 infections using PCR prior to the start of the experiment, and only uninfected lobsters were included, except when the experimental protocol required the use of infected lobsters, which all had visible signs (milky hemolymph) of disease observed through the dorsal membrane between the abdomen and cephalothorax. Therefore, all infections detected in lobsters at the conclusion of an experiment were new infections or infections that were initially well below the detection limit of the PCR test, which is quite low at 1.2 fg of purified viral DNA per positive test (Montgomery-Fullerton et al. 2007).

PaV1 prevalence among lobsters in the Florida Keys fishery

To determine the prevalence and distribution of PaV1 in the Florida (USA) fishery, we traveled aboard commercial lobster boats throughout the Florida Keys and sampled sublegal and legal lobsters collected in traps. We used a stratified-random sampling design, with the strata being four biogeographic regions in the Florida Keys: (1) middle Keys, Ocean-side, (2) middle Keys, Gulf-side, (3) lower Keys, Ocean-side, and (4) lower Keys, Gulf-side (Fig. 1). Within each stratum, we sampled lobsters from a haphazardly selected subset of traps. We conducted surveys near the beginning (August–November) and end (January–March) of the 2008–2009 fishing season. This seasonal sampling was im-

Fig. 1. Map of the Florida Keys (USA) showing the geographic regions where commercial traps were sampled (Gulf of Mexico, Atlantic Ocean, middle and lower Keys). The solid rectangle represents the region in the middle Keys where experimental traps were deployed.



portant to determine if temporal trends existed in trap catch and disease dynamics that may be associated with lobster density, trap soak times, or environmental correlates such as water temperature. We hypothesized that the effects of trap confinement on PaV1 prevalence, if present, would be more severe late in the fishing season when fishermen pull their traps less frequently.

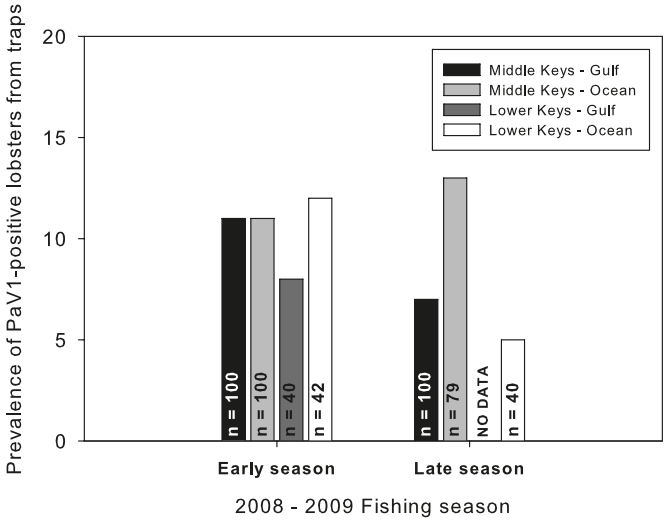
While on board, we obtained a haphazard sample of lobsters from each trap line, recorded the sex, injuries, and CL of each lobster, and then drew 0.2 mL of hemolymph with a 27-gauge syringe from the juncture between the basis and ischium of the fifth walking leg of each lobster. The hemolymph was stored in labeled microcentrifuge tubes filled with 0.6 mL of anticoagulant, held in an ice bath onboard the vessel, and then transferred to a –40 °C freezer until final shipment for PCR screening. Hemolymph samples were shipped on dry ice to the Virginia Institute of Marine Science for PCR analysis. The relationship between PaV1 prevalence and four independent variables (geographic region, stage of fishing season, sex, and size) was analyzed with logistic regression.

Effect of PaV1 infection on trap catch

To determine the effect of infected lobsters on trap catch, we manipulated the disease state (uninfected or infected) of sublegal lobsters normally used to bait commercial lobster traps and measured the "attractiveness" of those traps to wild lobsters. All experiments were conducted from May–July 2009 when the fishery was closed. The lobsters used in this experiment and the one described below were collected from hard-bottom and coral reef habitat around the middle Florida Keys, Florida, USA (Fig. 1), in the same areas where the traps were later deployed so as not to introduce additional infected lobsters into the wild. The status of infected and uninfected lobsters used in each experiment was determined by PCR prescreening of hemolymph samples for PaV1. All infected lobsters used as experimental bait displayed milky hemolymph, indicative of heavy infection, shown to elicit an avoidance response in conspecifics (Behringer et al. 2006).

Treatments were standard commercial fishing traps containing either a single uninfected ($n = 51$) or infected experimental lobster ($n = 33$) that served as "bait" in the trap (i.e., provided a social cue to other lobsters). The mean (± standard deviation, SD) size of infected experimental lobsters was 32.8 ± 9.8 mm, whereas that of uninfected experimental lobsters was 47.7 ± 11.8 mm. The prevalence of PaV1 in the wild is higher among smaller lobsters, hence the lower mean size of infected lobsters in our experiment (Shields and Behringer 2004). However, there is no relationship between the avoidance response of healthy lobsters and the size of either infected lobster or healthy lobsters (Behringer 2003). Each experimental lobster was constrained in a plastic minnow trap placed within the lobster trap to eliminate the possibility of their escape, but the traps were otherwise left unaltered. Traps were deployed in 2–5 m of water and then pulled 7 days later with the assistance of a commercial fisherman. We followed the advice of the fisherman in the haphazard placement of the traps in his normal fishing area. Traps were deployed either 500 m north of Long Key, Florida, or north of the Channel No. 5 bridge east of Long Key (Fig. 1), and at least 100 m apart. The mean number of uninfected lobsters that entered the traps was compared between experimental treatments using a one-way model I analysis of variance (ANOVA).

Fig. 2. Prevalence of PaV1 in Caribbean spiny lobsters (*Panulirus argus*) sampled from traps fished in four geographic regions in the middle and lower Florida Keys. There were no data collected from the Gulf of Mexico side of the lower Keys late in the season because the fishermen had removed their traps from this region. n = number of lobsters sampled from each region during the early and late stages of the fishing season.



Effect of trap confinement with an infected lobster on PaV1 transmission

We determined if confinement, or the stress associated with trap confinement, enhanced PaV1 transmission by sealing three uninfected lobsters in traps with either an uninfected lobster or a visibly infected experimental lobster. Lobsters were all marked with unique color-coded antennae tags for later identification. The mean (± SD) size of infected experimental lobsters was 37.2 ± 9.0 mm, whereas that of uninfected experimental lobsters was 43.3 ± 9.6 mm. After the experimental lobsters were added to standard wood-slat commercial lobster traps, we sealed the traps by covering the entire trap with 5 mm plastic mesh secured with stainless-steel staples. This prevented the experimental lobsters from escaping and also prevented any wild lobsters from entering the trap. Traps containing infected or uninfected experimental lobsters were haphazardly deployed at least 100 m apart from May–July 2009 using a commercial lobster boat in the middle Florida Keys (Fig. 1) as described above, and deployment periods alternated between 7 and 14 days. These durations spanned the range of soak times used in the fishery. Soak times are typically extended as the lobster season progresses and the legal lobster abundance decreases. The frequency with which uninfected lobsters became infected with PaV1, relative to experimental treatment and soak time, was analyzed using a logistic regression.

Results

PaV1 prevalence among lobsters in the Florida Keys fishery

Of the 502 lobsters sampled from commercial lobster traps throughout the Florida Keys, none had visible signs (milky hemolymph) of infection of PaV1, but 11% tested positive by PCR for the presence of PaV1 viral DNA in their hemolymph. The prevalence of infected lobsters varied little among geographic regions or between the early- and late-season samples (Fig. 2; Table 1). There was no significant relationship between region, season, sex, and prevalence of PaV1 (Table 2). For logistical reasons, no samples were acquired from the Gulf side of the lower Keys late in the fishing season.

Table 1. Demographic and seasonal distribution of Caribbean spiny lobsters (*Panulirus argus*) infected with PaV1 (PCR positive) and the resulting prevalence during the 2008–2009 fishing season.

Category	PaV1 infected (PCR+)	Uninfected (PCR–)	Total	Prevalence of PaV1 (%)
Sex				
Male	23	208	231	10.0
Female	26	244	270	9.6
Season				
Early	30	252	282	10.6
Late	19	200	219	8.7
Size				
Legal	18	133	151	8.8
Sublegal	31	319	350	11.9

Table 2. Effect of fishing season, geographic location, and sex on PaV1 prevalence in Caribbean spiny lobsters (*Panulirus argus*) caught in the Florida Keys commercial trap fishery.

Overall model evaluation			
Test	df	Likelihood ratio X^2	P
Logistic regression	5	2.0417	0.8433
Goodness-of-fit test			
Test	df	Pearson's X^2	P
Goodness-of-fit	5	2.042	0.4924
Effect likelihood tests			
Source	df	Likelihood ratio X^2	P
Fishing season	1	0.7219	0.3955
Geographic location	3	1.479	0.6871
Sex	1	0.06021	0.8062

The mean CL (±1SD) of lobsters sampled from traps was 75.9 ± 6.0 mm (maximum = 95.3 mm) for infected lobsters and 73.9 ± 6.2 mm (maximum = 109.3 mm) for uninfected lobsters. Because smaller lobsters are more susceptible to PaV1 (Shields and Behringer 2004; Butler et al. 2008), we analyzed the effect of size (CL) on the number of infected lobsters sampled from traps. There was a weak (P = 0.0508) negative relationship between lobster CL and prevalence of PaV1 (Table 3). When lobsters were grouped into legal (≥76 mm CL) or sublegal (<76 mm CL) sizes, there was no significant difference in the number infected (Fisher's exact test, likelihood X^2 = 1.086, df = 1, P = 0.3256).

Effect of PaV1 infection on trap catch

Traps deployed with an uninfected or PaV1-infected lobster all caught wild lobsters, but not equally (Fig. 3). The

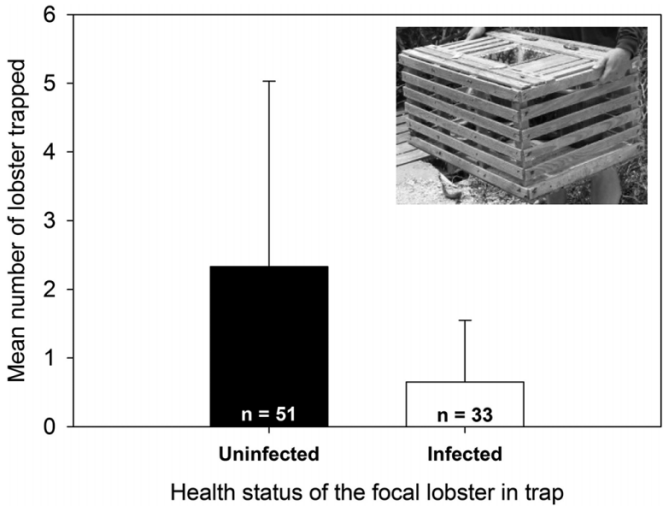
Table 3. Effects of lobster carapace length and fishing season on prevalence of PaV1 in Caribbean spiny lobsters (*Panulirus argus*) in the commercial trap fishery in the Florida Keys.

Overall model evaluation			
Test	df	Likelihood ratio X^2	*P*
Logistic regression	2	4.377	0.1120
Goodness-of-fit test			
Test	df	Pearson's X^2	*P*
Goodness-of-fit	2	206.64	0.9975
Effect likelihood tests			
Source	df	Likelihood ratio X^2	*P*
Carapace length	1	4.354	0.0508
Fishing season	1	0.02396	0.8770

Table 4. Effect of experimental Caribbean spiny lobster (*Panulirus argus*) health status and trap soak time on PaV1 transmission within experimental lobster traps.

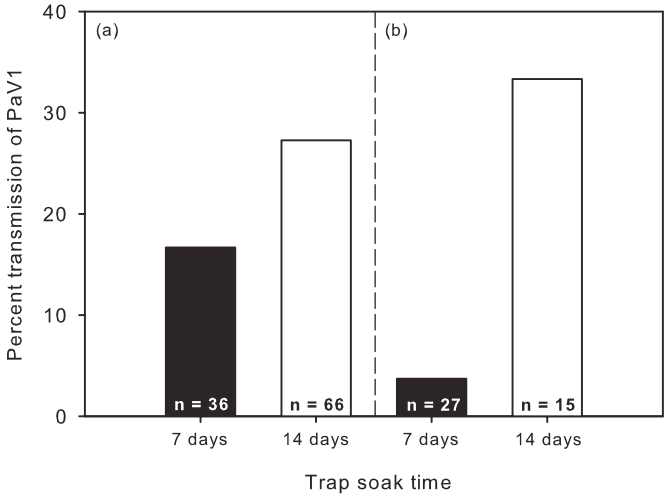
Overall model evaluation			
Test	df	Likelihood ratio X^2	*P*
Logistic regression	2	9.448	0.009
Goodness-of-fit test			
Test	df	Pearson's X^2	*P*
Goodness-of-fit	2	0.750	0.3863
Effect likelihood tests			
Source	df	Likelihood ratio X^2	*P*
Experimental lobster status	1	5.753	0.0165
Trap soak time	1	1.0156	0.3136

Fig. 3. Difference in catch in relation to the presence of an uninfected or infected experimental lobster. The mean (±1 standard deviation, SD) number of lobsters captured in traps baited with an uninfected lobster was significantly higher than that baited with a PaV1-infected juvenile lobster (one-way model I analysis of variance (ANOVA): $F = 4.2375$, df = 1,83, $P = 0.0427$). n = number of trap deployments within each treatment. Inset: Picture of a standard wood slat lobster trap used in the Florida fishery for Caribbean spiny lobster (*Panulirus argus*).



data were natural log transformed to meet the ANOVA assumption of normality, and the Welch ANOVA statistic was used because the variances were not homogeneous. The mean (± SD) number of lobsters captured by traps baited with an uninfected lobster was significantly greater (2.33 ± 2.7) than that for traps baited with a visibly PaV1-infected lobster (0.65 ± 0.9) (one-way model I Welch ANOVA: $F = 4.6621$, df = 1,78; $P = 0.0339$). That is, the traps with an infected juvenile lobster caught 72% fewer lobsters than those using uninfected bait animals. The mean (± SD) size of lobsters captured in the traps was 66.0 ± 10.0 mm for traps baited with an uninfected lobster and 62.6 ± 10.4 mm for traps baited with an infected lobster.

Fig. 4. Incidence of Caribbean spiny lobsters (*Panulirus argus*) newly infected with PaV1 when confined in traps with (*a*) a PaV1-infected experimental lobster or (*b*) an uninfected experimental lobster. n = number of lobsters within each treatment.



Effect of trap confinement with an infected lobster on PaV1 transmission

Transmission of the virus occurred within the traps. Significantly more uninfected lobsters became infected when confined with a PaV1-infected lobster than those confined with an uninfected lobster (Table 4; Fig. 4). Transmission that occurred during the 7-day trap soak time largely drove this difference, because after a 14-day soak, there was little difference in transmission between the infected or uninfected lobster treatments. In the control treatment, only one (<5%) of the 27 uninfected lobsters became infected with PaV1 after 7 days in a trap with another uninfected lobster, but after 14 days, 33% of the 15 control lobsters tested positive for PaV1 infection.

Discussion

PaV1 may have a profound effect on the Florida trap fishery for Caribbean spiny lobsters. The prevalence of PaV1

was 9% in legal-sized adult lobsters and 11% among all trapped lobsters. This finding is much higher than previously thought, but confirms findings of Shields and Behringer (2004) and Huchin-Mian et al. (2009) that adult lobsters can harbor infections. Traps baited with infected lobsters had lower catches than those baited with uninfected lobsters, which can be explained by the avoidance behavior of healthy lobsters towards diseased animals (Behringer et al. 2006). This has important consequences to the fishery, because in years or locales with high prevalence, catches may be lower if diseased lobsters enter them. We also found that infected lobsters can transmit PaV1 to uninfected lobsters in traps, although background infections were also high. This has important implications to the lobster fishery because it suggests that certain fishery practices could exacerbate the spread of the PaV1 virus.

Lobsters collected that were PCR-positive for PaV1 showed no visible signs of infection, but they were not removed from the fishery for histological examination to detect active infection. Visible infections with PaV1 are most commonly observed in small juveniles (Shields and Behringer 2004), and it is possible that these individuals escaped the traps as they were brought to the fishing boat. The PCR-positive individuals may also have had latent infections, or disease may manifest differently in the adult segment of the population resulting in infected animals that are not visibly diseased.

Avoidance by uninfected lobsters of infected lobsters is an effective means of reducing transmission of PaV1 (Behringer 2003; Behringer et al. 2006; Dolan 2011); thus uninfected lobsters may avoid traps containing infected lobsters. In the Florida fishery, traps are typically baited with sublegal lobsters, not food, thereby representing shelter in this shelter-limited system (Butler and Herrnkind 1997), and shelters containing conspecifics are attractive to other lobsters (Eggleston and Lipcius 1992; Ratchford and Eggleston 1998; Behringer and Butler 2006). However, not all uninfected lobsters avoided traps with visibly infected lobsters; they may be making a trade-off between avoiding disease versus avoiding predation (Lozano-Álvarez et al. 2008). Occasional observations of cohabitation by infected and uninfected lobsters in large, artificial shelters ("casitas") used by fisherman in Mexico were attributed to the large size of the casita relative to most natural crevice shelters within which lobsters are more closely spaced (Lozano-Álvarez et al. 2008). This may allow the lobsters to cohabitate with limited physical contact and may also apply to traps used in Florida. Perhaps more important to the interactions between infected and uninfected lobsters is the status and progression of disease in infected individuals and thus the timing and presence of signals that they produce and to which uninfected lobsters respond.

The interaction between fishery and pathogen has precedence in both crustacean and finfish fisheries (for several examples in crustacean fisheries, see Shields 2011*b*). Many reports of disease are associated with the holding of wild-caught organisms in impoundments. Pacific herring (*Clupea pallasi*) have a much greater prevalence (60%–87%) of viral hemorrhagic septicemia virus (VHSV) following their introduction to net pens in Prince William Sound, Alaska, presumably from exposure to free viral particles released from infected fish in the pen (Hershberger et al. 1999). Gaffkemia, caused by the bacteria *Aerococcus viridans* var. *homari* and Bumper Car Disease, caused by the ciliate *Anophryoides haemophila*, cause mortality of the American lobster *Homarus americanus* in impoundments (Snieszko and Taylor 1947; Stewart 1993; Greenwood and Cawthorn 2005). Some researchers have suggested that the baitfish used to trap *H. americanus* is of insufficient nutritional quality but is used so extensively (70%–80% of the lobster diet; Grabowski et al. 2005) that lobsters may be predisposed to chitinoclastic shell disease due to malnutrition and stress (Tlusty et al. 2008). Sablefish (*Anoplopoma fimbria*), often released as bycatch in the North Pacific, show impaired immune system function when subject to "experimental capture" in the laboratory (Lupes et al. 2006), potentially increasing the risk of infection and bycatch mortality. Fisheries and disease can also interact via the catchability of the host. Many parasites affect the behaviors of their host, altering predation susceptibility (Minchella and Scott 1991; Bakker et al. 1997; Hall et al. 2005). For example, when the Norway lobster (*N. norvegicus*) are heavily infected with the pathogenic dinoflagellate *Hematodinium* sp., they have a much reduced escape response and are more apt to be captured in trawls relative to healthy lobsters (Stentiford et al. 2001). However, differences in trap and trawl efficiency or entry can vary markedly in other hosts infected by *Hematodinium* sp. (Wilhelm and Mialhe 1996; Pestal et al. 2003; Shields et al. 2005). Fisheries and disease can also have indirect effects on populations as shown in the California spiny lobster (*Panulirus interruptus*) fishery in California (USA). In marine reserves, where lobster fishing is prohibited, density-dependent disease from *Vibrio* sp. is rare among lobster prey, the purple urchin (*Strongylocentrotus purpuratus*), but in fished areas, urchin density grows unchecked and epizootics are frequent (Behrens and Lafferty 2004).

Our results show that fishery practices also have a direct effect on disease dynamics in *P. argus*. The use of sublegal lobsters in traps greatly increases their mortality (Kennedy 1982; Hunt et al. 1986; Hunt 2000), and our study shows that trap confinement can also result in PaV1 transmission. Stress increases susceptibility to disease in finfish (Davis et al. 2002; Huntingford et al. 2006), so stress-induced confinement could potentially increase the risk of PaV1 infection in spiny lobsters. Transmission of PaV1 by contact and ingestion of infected food (i.e., via cannibalism) (Butler et al. 2008) may also be enhanced among lobsters confined in traps with limited food. Moreover, even without an infected lobster within the trap, infection is possible from an endemic source of PaV1, and this may increase with longer soak times.

This source is unidentified, but it could well be the presence of diseased lobsters in the surrounding habitat from which the trapped lobsters cannot escape. If true, it may be that waterborne transmission is more efficient than initially determined from histological examination of experimental lobsters (Butler et al. 2008). It is unlikely that transmission occurred between our experimental traps containing infected lobsters and those containing only uninfected lobsters because we never placed more than a single infected lobster in each trap and traps were far from one another (>100 m). Thus, a more plausible source of infection was natural "background" sources from other infected lobsters in the area or

perhaps an unidentified prey item acting as a pathogen reservoir. Regardless of the source, sublegal lobsters used in traps and released by fishermen after use are more likely to be infected with PaV1 if confined with infected lobsters.

Managing PaV1 in the fishery

Managing disease in fisheries is notoriously difficult because the disease dynamics often occur outside the act of capture. Typical solutions include reducing crowding in impoundments, careful handling of sublegal organisms or bycatch, and prohibiting the discard of diseased individuals back into the water (Shields 2003; Shields and Overstreet 2007). In the Florida trap fishery, more careful use of sublegal bait lobsters may reduce the spread of PaV1. Lobstermen should (*i*) remove from the water and destroy any lobsters with obvious signs of infection (i.e., milky hemolymph observed between the abdomen and cephalothorax), (*ii*) keep trap soak times to a minimum (<7 days), (*iii*) not move sublegal lobsters from one region to another, and (*iv*) not hold sublegal lobsters under crowded conditions in tanks or cages. Reducing the confinement time of sublegal lobsters in traps will reduce their mortality and perhaps their stress and risk of infection by PaV1. The use of alternative methods or gear should also be evaluated. For example, dive-based fisheries that do not include the use of artificial habitats (e.g., casitas) may minimize the risk of PaV1 infection if they do not artificially concentrate lobsters. Alternatively, leg and antennae injuries are more common in dive-based fisheries and that may increase the risk of infection.

Fishery trends and PaV1

The drivers of fishery stock dynamics are often multifaceted or unknown, and such is the situation with *P. argus*. Although there is no doubt that PaV1 can be a major source of juvenile lobster mortality where the disease is prevalent, there are no data prior to 2000 with which to determine the time of its emergence. Along the Yucatan coast of Mexico, prevalence of PaV1 in juveniles has increased from 2.7% in 2001 to 10.9% in 2006 (Lozano-Álvarez et al. 2008). However, annual surveys conducted since 2000 in the Florida Keys indicate that prevalence of visibly infected juveniles has changed little, fluctuating between 2% and 8% (Behringer et al. 2011). During the same period, lobster landings plummeted ~30% and have remained low (SEDAR8 2010).

Indeed, recent commercial fishery landings of *P. argus* have declined in most Caribbean nations, and most stocks are considered overexploited (Chávez 2009; Ehrhardt et al. 2010). Although the importance of overfishing on postlarval production is obvious, in Florida, there are a number of environmental perturbations that could further impact the fishery in ways similar to PaV1, including harmful algal blooms (Phlips et al. 1999), seagrass die-offs (Rudnick et al. (2005) and references therein), sponge die-offs (Butler et al. 1995; Peterson et al. 2006), and declining water quality (Boyer and Briceño 2005). However, they do not correspond with the discovery of PaV1 or the local decline in fishery landings and do not explain similar downturns elsewhere in the Caribbean. Given our findings, additional studies on the prevalence of the virus in populations of the Caribbean spiny lobster are warranted.

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