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Spatial dynamics in the social lobster *Panulirus argus* in response to diseased conspecifics

Joshua R. Anderson[1,2], Donald C. Behringer[1,2,*]

[1]School of Forest Resources and Conservation, Program of Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida 32653, USA

[2]Emerging Pathogens Institute, University of Florida, Gainesville, Florida 32611, USA

ABSTRACT: Pathogens can influence the behaviors of their hosts, including avoidance by healthy individuals. However, the spatial structuring consequences of these behaviors are often unknown, especially in the marine environment. The Caribbean spiny lobster *Panulirus argus* (Latreille, 1804) avoids sheltering with conspecifics infected with *P. argus* virus 1 (PaV1). We sought to determine the behavioral cue and if this behavior alters population spatial structure in the wild. We used a series of laboratory Y-maze experiments to determine the mechanism of detection and also employed artificial shelter arrays placed in different flow conditions in the field to determine the effect of diseased lobster aversion on healthy lobster spatial dynamics. Results showed that avoidance is driven by the chemoreception of cues based in the urine. The chemical cue alone was as effective as having a diseased lobster present and visible. The presence of single PaV1-infected lobsters altered the small-scale spatial structure of wild populations, especially under low flow regimes, via redistribution of neighboring individuals. In shelter-limited environments, disease avoidance has the potential to increase juvenile mortality, as nearby conspecifics are displaced from dens and exposed to a higher risk of predation.

KEY WORDS: Caribbean spiny lobster · Chemoreception · Pathogen · *Panulirus argus* virus 1 · PaV1 · Population structure

INTRODUCTION

Pathogens are known to affect the behavior of their hosts, including avoidance by healthy individuals (Dugatkin et al. 1994, Kavaliers & Colwell 1995, Behringer et al. 2006). In sessile marine organisms such as scleractinian corals and gorgonians, disease can spatially structure populations by depleting local areas of the infected individuals (Aronson & Precht 2001, Dube et al. 2002, Richardson & Voss 2005, Toledo-Hernández et al. 2007) — a logical effect for organisms that cannot escape disease. Although there is some evidence that disease avoidance can affect the structure of terrestrial and aquatic populations (Ban et al. 2008, Tobler & Schlupp 2008), only anecdotal information exists for this effect in mobile marine organisms (Sousa 1991, Thompson et al. 2005, Behringer et al. 2006).

The detection of conspecifics and presumably disease typically occurs through chemosensory or visual signals. Among the former, highly soluble signals make aquatic chemoreception highly efficient and results in it being used extensively for inter- and intraspecific communication (Díaz & Thiel 2004, Larson & McCormick 2005). For example, mate searching by the female rock shrimp *Rhynchocinetes typus* is mediated predominantly by chemical cues (Díaz & Thiel 2004). Chemoreception of urine drives many decapod behaviors (Bushmann & Atema 1997, Karavanich & Atema 1998) due to its high concentration of

*Corresponding author. Email: behringer@ufl.edu

soluble proteins (Atema & Steinbach 2007). The concentration of proteins and other inherent wastes within the urine can be indicative of the overall health of an organism (Breithaupt & Eger 2002), making it a logical indicator of disease.

Other urine-regulated behaviors, such as aggregation, can have population-structuring effects by influencing predation rates (mysids, *Tenagomysis* sp.: O'Brien & Ritz 1988; Caribbean spiny lobster *Panulirus argus*: Childress & Herrnkind 2001) or providing reproductive refuge for vulnerable mates (American lobster *Homarus americanus*: Bushmann & Atema 1997). When the structuring effects of aggregation are coupled with disease avoidance, infected conspecifics could further influence spatial structure. However, hydrological turbulence and dilution of chemical cues may interfere with chemical detection and subsequent aggregation (Weissburg & Zimmer-Faust 1993, Finelli 2000, Jackson et al. 2007, Page et al. 2011). As flow velocity increases, odor plume concentration decreases, and physical structures (e.g. sponges, octocorals, and seagrass beds) downstream of the odor source turn a single odor plume into many small, dilute plumes (Finelli 2000, Jackson et al. 2007), causing a reduction in chemoreception efficiency (blue crab *Callinectes sapidus*: Weissburg & Zimmer-Faust 1993). Thus, behaviors mediated by chemical cues could be correlated to local hydrodynamics that could play a factor in the detection range and strength of the chemical cue.

Chemoreception is clearly important in structuring marine populations, but visual senses are also well adapted in crustaceans that inhabit shallow, well-lit environments (Acquistapace et al. 2002, Van der Velden et al. 2008, Baldwin & Johnsen 2009). For instance, spiny lobsters use visual signals to align themselves in a linear formation during diurnal migrations (Herrnkind 1969). Likewise, Baldwin & Johnsen (2009) found that mate choice in blue crabs could be stimulated based exclusively on visual signals, although chemical cues are very important in blue crab courtship behavior in a natural setting (Kamio et al. 2008) and could be used together. Visual signals in concert with chemical cues (i.e. bimodal cues) are commonly required in nature to induce a behavioral response in decapods (Acquistapace et al. 2002, Raethke et al. 2004). For example, Raethke et al. (2004) demonstrated that the female southern rock lobster *Jasus edwardsii* uses bimodal cues to identify possible reproductive partners. Bimodal communication plays a role in intraspecific interactions, but it also structures populations by mediating the aggregation of conspecifics or possibly disease detection.

The Caribbean spiny lobster *Panulirus argus* (Latreille, 1804) is a normally gregarious decapod whose social behaviors are driven by chemosensory and visual signals. As juveniles, they are commonly infected with a pathogenic virus *Panulirus argus* virus 1 (PaV1), which causes visual signs of 'milky' white hemolymph and a reddening of the carapace in overtly infected individuals (Shields & Behringer 2004). The prevalence of this virus is decreased by the ability of uninfected lobsters to detect and avoid infected conspecifics and the dens they inhabit (Behringer et al. 2006, 2011). Diseased den avoidance presumably limits transmission (Behringer et al. 2006), but also effectively removes those dens from the suite available and could potentially alter the spatial structure of the population. This effect could be especially pronounced and lead to increased exposure to predation (Behringer & Butler 2010) when disease prevalence is high or shelter is limited. Possible implications of disease avoidance in a shelter-limited habitat could be the movement and redistribution of healthy individuals away from infected conspecifics, which in turn could influence predation and transmission rates.

We used the Caribbean spiny lobster as a model organism to investigate the ecological effects of disease avoidance on spatial structuring dynamics. We used a series of experiments to determine: (1) the cue lobsters use to detect PaV1-infected lobsters, (2) the source of the cue, (3) if PaV1 affects spatial structure, and (4) if disease detection and spatial structuring dynamics are correlated with high or low flow regimes.

MATERIALS AND METHODS

Lobsters

For the 3 Y-maze laboratory experiments, juvenile *Panulirus argus* were collected by hand-net from hard-bottom habitat (<3 m depth) 1 to 3 km north of the middle Florida Keys (USA) during the summer of 2010. Lobsters (25 to 50 mm carapace length, CL) were held in UV-treated flow-through tanks under a natural photoperiod and fed shrimp ad libitum. Thirty of these lobsters were inoculated with PaV1 by the process described in Behringer et al. (2008) and held separately from non-diseased animals in recirculating tanks.

General Y-maze experimental design

Laboratory experiments were conducted in four 80 l Y-mazes (94 cm long × 62 cm wide × 20 cm tall, with a 72 cm long × 18 cm tall central divider) constructed of epoxy-coated plywood (Fig. 1). This Y-maze size provided an efficient experimental setup to determine the influence of disease on small non-diseased lobsters over a short distance. Each upstream arm of the Y-maze held conditioned brick shelters through which unfiltered, ambient seawater (mean ± SD temperature: 25 ± 1°C; salinity: 35 ± 1) was gravity fed from isolated 120 l head tanks at a rate of 4.0 ml s^{-1} (Fig. 1). Water flowed from the shelters at a rate of 1.0 cm s^{-1}, through the arms of the Y-maze, and drained out of a standpipe at the base of the Y-maze. Flow rate was controlled via labcock valves 6.35 cm from the bottom of the head tanks and monitored temporally by flow into a graduated beaker (1 l) daily. Dye tests confirmed that water from each Y-maze arm was unidirectional and restricted to its respective arm before converging at the base of the Y-maze. All Y-maze experiments were conducted for 8 h at night when *Panulirus argus* is most active, and ended 1 h post-dawn, after lobsters have sought their diurnal shelter (Zimmer-Faust et al. 1985). Likewise, this experimental duration allowed the subjects to, under their natural photoperiod, choose a final shelter pre- to post-dawn, when they actively search for shelters inhabited by conspecifics, and more specifically, actively following cues emitted by these conspecifics (the 'guide effect'; Childress & Herrnkind 2001). In all Y-maze experiments, treatments were randomly assigned (coin flip) to each head tank of the Y-maze 4 h prior to the start of the trial to infuse the water with that odor. Placing the treatments randomly helped correct for slight variations in flow rate by ensuring that, over the course of the experiment, subjects would experience these slight variations coming from both treatments. At the onset of the trial, a visibly uninfected lobster (clear hemolymph; Shields & Behringer 2004) was measured (CL; to the nearest 0.1 mm), sexed, and positioned at the base of the Y-maze behind a porous screen. Lobsters were given a 5 min 'calming period', after which the screen was removed and the lobster was allowed to move about freely. Preliminary observations (n = 12) indicated that lobsters approached each branch of the Y-maze, thus receiving cues from each. At the end of each trial, the shelter treatment that the focal lobster selected was recorded. Between trials, each Y-maze system was flushed with fresh water and allowed to air dry for 10 h.

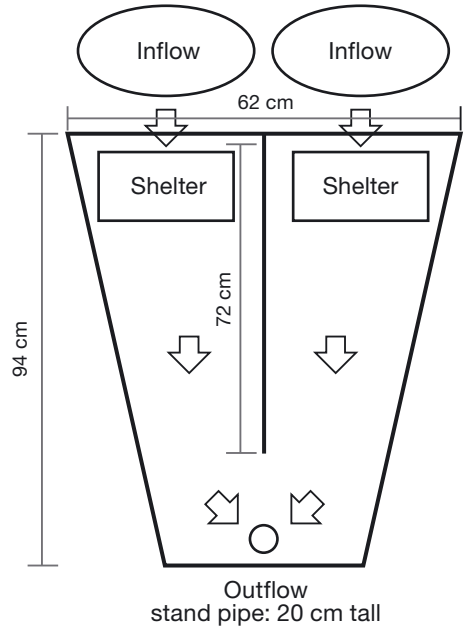

Fig. 1. *Panulirus argus* virus 1 (PaV1) avoidance. Y-maze design used for all Y-maze experiments. During the visual signal experiments, a glass divider was installed 6 cm from the upstream end of the Y-maze, and the attracting shelters were moved downstream of this glass divider with water released through these shelters as in the chemical cue experiments

To determine if the sheltering choice deviated from random for all Y-maze experiments mentioned below, a 2-tailed binomial with the probability of sheltering set at 50% (random sheltering) was used. Significance was determined at $\alpha = 0.05$. Unsheltered (n = 4) or escaped lobsters were neglected from data analysis.

Expt 1: chemosensory detection of PaV1-infected conspecifics

To determine if *Panulirus argus* could use chemoreception alone to detect and avoid PaV1-infected conspecifics, 3 comparisons were made. The first tested the chemical cues emitted from visibly uninfected lobsters in one head tank versus no cue (seawater only) emitted from the second head tank (n = 30). In the second comparison, the chemical cues emitted from visibly diseased lobsters in one head tank were tested versus no cue (seawater only) emitted from the second head tank (n = 20). Finally, to determine if lobsters favored the odor of healthy conspecifics over visibly infected conspecifics, visibly uninfected lobster chemical cues were tested versus the chemical cues from visibly infected lobsters (n = 21).

Expt 2: source of chemical disease cue

To test if PaV1 was detected chemically via urine, Y-maze experiments were conducted with the nephropores of diseased lobsters blocked (n = 30). In spiny lobsters, the nephropores are the main urine-release sites, located ventrally below the antennae (Shabani et al. 2009). The nephropores of the diseased lobsters were blocked by blotting the nephropores dry and then covering them with 2 coats of cyanoacrylate gel (Krazy Glue®), followed by a thorough inspection to insure complete coverage and elimination of fluid release. To reduce experimental bias, the same procedure was carried out on control lobsters (non-diseased) except that no glue was applied on the nephropores. After application of the plug, diseased lobsters were placed in a head tank while non-diseased lobsters (unblocked nephropores) were placed in the other head tank 4 h prior to each trial. The nephropore plugs were removed at the end of the trial, and all lobsters survived.

Expt 3: visual detection of PaV1-infected conspecifics

To determine if lobsters could visually detect PaV1-infected individuals, we modified the Y-mazes by installing a sheet of glass (18 cm high × 28 cm wide) 6 cm in front of each upstream section of the Y-maze and secured it with silicone caulking to eliminate water exchange. The procedure was the same as described in Expt 1, except that no lobsters were present in the head tanks, treatment lobsters were placed upstream of the glass partitions, and the seawater was released downstream of the glass partition through the attracting shelters. Thus, there was a visual signal, but the only chemical cue was seawater. This experiment included 3 comparisons: visibly uninfected lobster visual signals versus no signal (n = 30), visibly diseased lobster visual signals versus no signal (n = 23), and visibly uninfected lobster visual signals versus diseased lobster visual signals (n = 30).

Field experiment: influence of PaV1 avoidance on juvenile population spatial structure

In fall 2010, a mark-recapture experiment was conducted 1 to 2 km north of the middle Florida Keys (USA) to assess the effects of PaV1 avoidance on spatial structure. The experiment employed artificial shelter arrays designed to take into account the chemosensory capabilities of *Panulirus argus* (Derby et al. 2001, Shabani et al. 2009; our Fig. 2). Eight shelter arrays were placed >100 m apart in hard-bottom habitat (~2 m depth), in areas devoid of natural shelters. The artificial shelters were composed of double-stacked concrete partition blocks conditioned for 3 mo in the ocean prior to use to make them similar in appeal to natural structures (Behringer & Butler 2006). To determine whether flow regime was correlated with population spatial dynamics, 4 arrays were placed in an environment described as 'low flow' (this flow rate was similar to our Y-maze flow rate; see 'Results') and 4 in an environment described as 'high flow.' These environments were determined from local knowledge of Florida Bay, in areas with very different flow regimes. To demonstrate that the environments had different flow regimes, the average daily flow (24 h) was calculated using a digital/mechanical flow meter (Model 2030, G.O. Environmental) with measurements taken 0.5 m from the ocean floor. This average daily flow rate does not take into account the actual complexities of turbulent flow, but simply indicated that there were differences between the 2 environments and allowed us to compare spatial structuring differences between flow regimes. This design allowed us to address 2 effects through time: the effect of a diseased lobster on the abundance of other lobsters (movement on or off the study site) within a defined radius, and the effect of a diseased lobster on the shelter choice of lobsters (i.e. redistribution) within a defined radius.

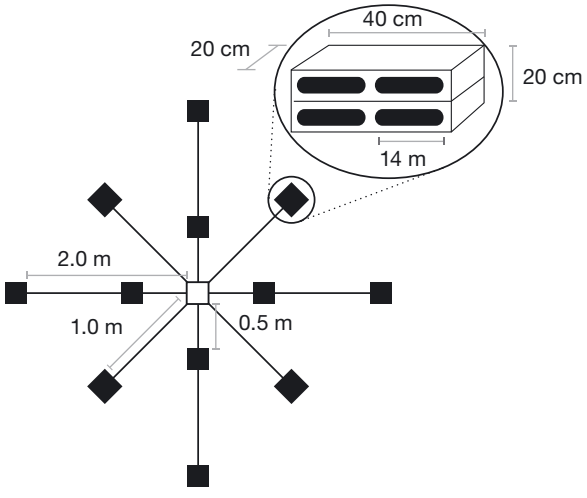

Fig. 2. *Panulirus argus* virus 1 (PaV1) avoidance. Geometric arrangement of artificial shelters used to determine the population spatial structuring effects of PaV1-infected lobsters. Black boxes: a set of double-stacked concrete block shelters; central white box: location of the treatment

The geometric formation of the shelters allowed multiple distances from the central shelter to be calculated: 0.5, 1, and 2 m (Berger & Butler 2001; our Fig. 2). Net movement was determined by adding movement towards and subtracting movement away from the treatment of each individual lobster (lateral movement = 0), while movement on and off the study site were subtracted or added to the total relative to the original abandoned shelter (off) or the newly inhabited shelter (on).

After colonization of the shelters (3+ lobsters per array), tagging surveys were conducted. Prior to dusk, 2 SCUBA divers used hand-nets to capture each lobster on the site, record its CL and shelter location, and mark it with a unique color-coded antenna tag before returning it to its respective shelter. After the initial surveys (t_0), a visibly non-diseased or diseased juvenile collected from nearby was tethered with a 20 cm piece of monofilament line to the central block in the shelter array (Fig. 2). Subsequent surveys were conducted at 24 h (t_{24}) and 48 h (t_{48}), during which divers recorded the location and tag code for each tagged lobster. New arrivals at t_{24} were treated as those at t_0. If tagged animals were encountered during the t_{24} survey, the antenna tag was recorded but the lobster was left undisturbed. A 1 mo time period separated each group of tagging replicates. Previous studies have indicated that crustaceans lose olfactory memory of previously encountered individuals after being separated for 2 wk in the American lobster *Homarus americanus* (Karavanich & Atema 1998), 2 wk in the common crayfish *Cherax destructor* (Hemsworth et al. 2007), and 1 wk in the rusty crayfish *Orconectes rusticus* (Zulandt Schneider et al. 2001). This amount of time should have been sufficient to erase the olfactory recognition of the treatment lobsters from animals that emigrated or remained on the study site (Karavanich & Atema 1998, Zulandt Schneider et al. 2001, Hemsworth et al. 2007).

Two separate repeated-measures ANOVAs were used (SAS 9.2, SAS Institute) to analyze the data due to unequal time factors between the 2 response variables, lobster abundance and shelter distance moved. Abundance was recorded thrice per site (t_0, t_{24}, t_{48}), while the distance moved could only be calculated twice, between t_0 and t_{24} and between t_{24} and t_{48}. Both ANOVAs included a within-subject term (time) and 2 between-subject factors (distance and disease). If a repeated-measures ANOVA was significant ($\alpha = 0.05$), Tukey-Kramer multiple comparison tests were used to determine the source of difference.

RESULTS

Y-maze experiments

Visibly uninfected treatment animals in the Y-maze experiments had a mean (± SD) CL of 34.1 ± 6.3 mm, while visibly diseased treatment animals had a mean CL of 31.3 ± 5.1 mm. Focal animals in the visibly uninfected versus seawater-only treatment selected the seawater-only shelters 67% of the time, but this did not differ from random ($n = 30$; $p = 0.0987$; $1 - \beta = 0.432$ [$1 - \beta$ = power of test; no predetermined benchmarks set]; Fig. 3). Focal lobsters in the visibly infected versus seawater-only treatment preferred the seawater-only shelters (76%) compared to diseased chemical cue shelters ($n = 20$; $p = 0.0414$; $1 - \beta = 0.617$). Likewise, focal lobsters selected shelters with non-diseased chemical cues 86% of the time when given the choice between diseased and non-diseased chemical cues ($n = 21$; $p = 0.0015$; $1 - \beta = 0.932$). When the nephropores of visibly infected lobsters were blocked, focal lobsters selected shelters supplied with water from head tanks containing visibly

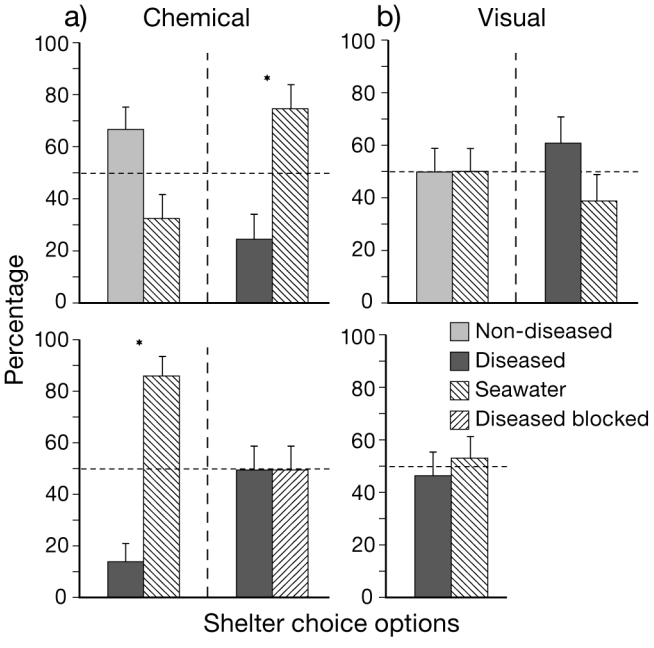

Fig. 3. *Panulirus argus* virus 1 (PaV1) avoidance. Shelter choice (%) of focal lobsters in Y-maze experiments. (a) Chemical sensing experiments in which no visual signals were present. (b) Visual experiments in which no chemical cues were present (non-diseased vs. seawater only: $p = 1.0000$, $1 - \beta = 0.043$; diseased vs. seawater only: $p = 0.4049$, $1 - \beta = 0.143$; diseased vs. non-diseased: $p = 0.8555$, $1 - \beta = 0.057$). Horizontal dotted line: random sheltering (50%). *Paired bars with an asterisk were significantly different ($p < 0.05$) from random. Error bars represent 1 SE

uninfected or visibly infected head tanks equally (50%), suggesting that urine is the source of the chemical cue (n = 30; p = 1.0000; 1 − β = 0.043). During visual experiments, no sheltering within the treatments was different than random (Fig. 3).

Influence of PaV1 avoidance on juvenile population spatial structure

During the 28 mark-recapture experiments (diseased: n = 12, non-diseased: n = 16; or high flow: n = 14, low flow: n = 14), 258 wild lobsters were tagged, with a mean (± SD) CL of 29.6 ± 7.5 mm. There was a mean density of 8.1 ± 4.8 wild lobsters per site during the t_0 surveys. The mean CL of visibly healthy tethered lobsters was 37.0 ± 6.4 mm, and 29.9 ± 3.9 mm for visibly diseased tethered lobsters. CL of tethered animals differed between treatments (*t*-test: df = 25.25; $t = -3.01$; p = 0.0058), but because diseased animals were smaller (Shabani et al. 2009), any bias would have been in favor of not finding a difference between diseased and non-diseased control treatments. Flow regimes were determined to be significantly different, with low flow sites (n = 3; flow rate: 0.20 cm s^{-1}; 95% CI: −0.23 to 0.63 cm s^{-1}) having a slower flow rate (df = 2.02; $t = 4.79$; p = 0.0403) than high-flow sites (n = 3; flow rate: 7.94 cm s^{-1}; 95% CI: 1.00 to 14.88 cm s^{-1}).

Experiments comparing the changes in lobster abundance over time indicated that health status (df = 146; $F = 11.42$; p = 0.0009) and time (df = 71; $F = 20.17$; p < 0.0001) differed from random (Table 1a). Although there was a trend of lobsters redistributing themselves away from the diseased lobsters, the number of lobsters on disease treatment sites did not differ between flow regimes (df = 146; $t = -2.40$; p = 0.0810; Table 2). Within the low flow treatment, there were significantly fewer lobsters present on disease treatment sites (df = 146; $t = -3.84$; p = 0.0010; Table 2). The number of lob-

Table 1. *Panulirus argus* virus 1 (PaV1) avoidance. Two repeated-measures ANOVAs used to determine the effect of PaV1-infected lobsters versus non-diseased controls on (a) abundance of lobsters per site over time and (b) distance moved away from the PaV1-infected lobster by neighboring conspecifics over time, under low- and high-flow regimes. ndf: numerator df; ddf: denominator df; t: time. *Significant (p < 0.05)

	(a) Abundance					(b) Distance moved				
Source	ndf	ddf	χ^2	*F*	p	ndf	ddf	χ^2	*F*	p
Time	2	71	40.33	20.17	<0.0001*	1	43	2.29	2.92	0.0946
Health status	1	146	11.42	11.42	0.0009*	1	290	17.82	17.82	<0.0001*
Flow	1	146	2.95	2.95	0.0879	1	290	0.04	0.04	0.8331
Distance (from treatment)	2	146	2.38	1.19	0.3069	2	290	182.6	91.3	<0.0001*
Health × Flow	1	146	3.63	3.63	0.0586	1	290	4.20	4.20	0.0414*
Health × Time	2	146	4.49	2.24	0.1097	1	290	1.44	1.44	0.2315
Flow × Time	2	146	1.68	0.84	0.4345	1	290	0.34	0.34	0.5629
Health × Distance	2	146	0.51	0.26	0.7734	2	290	19.41	9.71	<0.0001*
Flow × Distance	2	146	7.20	3.60	0.0298*	2	290	1.09	0.54	0.5812
Time × Health × Distance	8	146	18.27	2.28	0.0248*	4	290	4.23	1.06	0.3777
Health × Flow × Distance	2	146	5.37	2.69	0.0715	2	290	4.30	2.15	0.1186
Within subject		SE	df	*t*	p		SE	df	*t*	p
	Time					Time				
	t_0	0.0410	71	38.51	<0.0001*	t_0–t_{24}	0.0826	43	1.79	0.0804
	t_{24}	0.0425	71	34.03	<0.0001*	t_{24}–t_{48}	0.0977	43	−0.55	0.5844
	t_{48}	0.0410	71	32.04	<0.0001*					
Between-subject		SE	df	*t*	p		SE	df	*t*	p
	Distance					Distance				
	0.5 m	0.0415	146	34.28	<0.0001*	0.5 m	0.1162	290	−8.79	<0.0001*
	1 m	0.0415	146	34.51	<0.0001*	1 m	0.1038	290	2.92	0.0038*
	2 m	0.0415	146	35.76	<0.0001*	2 m	0.0959	290	8.96	<0.0001*
	Health					Health				
	Diseased	0.0503	146	26.53	<0.0001*	Diseased	0.1115	290	−2.16	0.0317*
	Non-diseased	0.0438	146	35.61	<0.0001*	Non-diseased	0.0792	290	4.22	<0.0001*

Table 2. *Panulirus argus* virus 1 (PaV1) avoidance. Tukey-Kramer multiple comparison tests used to determine the spatial structuring effects of PaV1 relative to the number of lobsters per site. (a) Compares the health status of treatment animals and flow regime. (b) Compares the initial proximity to the central treatment shelter in the artificial array and health status. Tukey-Kramer adjusted p is the p-value adjusted for the multiple pairwise comparisons. *Significant ($p < 0.05$)

Within	Between	ddf	Difference of means	SE	*t*	Adjusted p
(a) Health status and flow regime						
Disease	Flow regime	146	−0.24	0.101	−2.40	0.0810
Non-diseased	Flow regime	146	0.01	0.088	0.14	0.9989
Low flow	Health status	146	−0.35	0.092	−3.84	0.0010*
High flow	Health status	146	−0.10	0.097	−1.02	0.7408
(b) Initial proximity and health status						
0.5 m	Health status	146	−0.25	0.083	−3.05	0.0314*
1.0 m	Health status	146	−0.23	0.083	−2.77	0.0690
2.0 m	Health status	146	−0.19	0.083	−2.32	0.1929

sters on non-disease treatment sites was not affected by the flow regime (df = 146; $t = 0.14$; p = 0.9989), nor did health status affect the number of lobsters on high flow sites (df = 146; $t = -1.02$; p = 0.7408). The health status of the tethered lobster also affected the number of lobsters located at different distances from the central shelter (Table 2). There were fewer lobsters located at 0.5 m from the central shelter on disease treatment sites compared to non-diseased treatment sites (df = 146; $t = -3.05$; p = 0.0314), but not at 1 m (df = 146; $t = -2.77$; p = 0.0690) or 2 m (df = 146; $t = -2.32$; p = 0.1929; Table 2). Analysis of the interaction of health status, flow rate, and distance from central shelter revealed that at a distance of 0.5 m in a low flow regime, there were fewer lobsters on disease treatment sites (df = 146; $t = -3.95$; adjusted p = 0.0066). No other interactions were statistically different from random.

The mean shelter distance moved by lobsters was affected by the health status of the tethered lobster (Table 1b, Fig. 4) and initial distance from central shelter (df = 290; $F = 91.30$; p < 0.0001), but flow regime was not a factor (df = 290; $F = 0.04$; p = 0.8331). Within the disease treatment, movement did not differ between low and high flow regimes (df = 290; SE = 0.223; p = 0.6741), nor did movement differ within the non-disease treatment (df = 290; SE = 0.157; p = 0.2091). Within the high-flow treatment, movement did not differ between the diseased and non-diseased treatment, but within low-flow sites, lobsters moved sig-

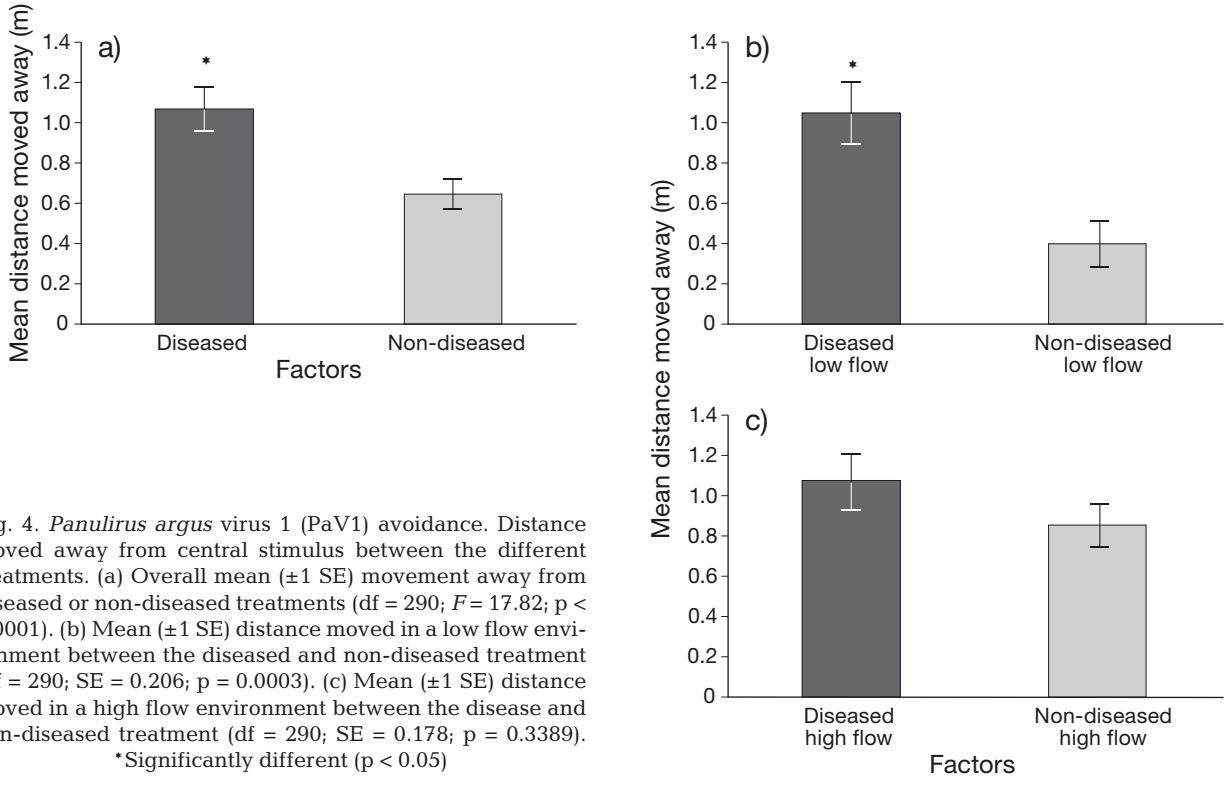

Fig. 4. *Panulirus argus* virus 1 (PaV1) avoidance. Distance moved away from central stimulus between the different treatments. (a) Overall mean (±1 SE) movement away from diseased or non-diseased treatments (df = 290; $F = 17.82$; p < 0.0001). (b) Mean (±1 SE) distance moved in a low flow environment between the diseased and non-diseased treatment (df = 290; SE = 0.206; p = 0.0003). (c) Mean (±1 SE) distance moved in a high flow environment between the disease and non-diseased treatment (df = 290; SE = 0.178; p = 0.3389). *Significantly different ($p < 0.05$)

nificantly further away from the central treatment shelter on disease treatment sites (Fig. 4). Within a given distance from the central treatment, lobster movement was also affected by the health status of the tethered lobster. At the 0.5 m distance, lobsters moved farther away from the diseased treatment (df = 290; SE = 0.231; $p < 0.0001$), while movement was not different at a distance of 1 m (df = 290; SE = 0.208; $p = 0.8325$) and 2 m (df = 290; SE = 0.191; $p = 0.9651$).

DISCUSSION

The presence of diseased individuals can potentially structure a marine population spatially, driving small-scale movement patterns, and may do this largely via chemoreception. We have shown that the avoidance behavior healthy lobsters display in response to PaV1-infected lobsters (Behringer et al. 2006) can be driven solely by chemical cues present in the urine of diseased individuals, and this behavior altered the spatial structure of juveniles in experimental field sites through redistribution away from diseased animals. The influence of local hydrodynamics complicated these results, with greater effects being correlated with low flow environments. The consequence of spatial redistribution may be a decrease of disease transmission in the wild (Behringer et al. 2011), but indirect effects (e.g. increased shelter search time) could raise predation rates where disease prevalence is high or shelter is limited (Behringer & Butler 2010).

Chemoreception is a highly efficient mode of habitat assessment in the marine environment and is used to find food (Zimmer-Faust 1993, Finelli et al. 2000), avoid predators (Larson & McCormick 2005), and mediate species-specific interactions (Díaz & Thiel 2004), and now we have demonstrated its use in avoiding disease among social lobsters. As with other intraspecific interactions driven by chemoreception (Zulandt Schneider et al. 2001, Díaz & Thiel 2004), urine detection alone regulated PaV1 avoidance. Lobsters selected dens emitting chemical cues from diseased and non-diseased lobsters equally when the nephropores were blocked. Moreover, visual signals alone were insufficient to cause avoidance of PaV1-infected lobsters, as focal lobsters did not avoid infected lobsters when only visual signals were present. Although not explicitly tested, chemoreception may decrease the need for visual detection or bimodal signal enhancement because it can still be used when visual signals are diminished (e.g. turbidity). Likewise, chemoreception may dominate the detection of PaV1 because it is sensitive to infected conspecifics prior to infection or infectiousness (Behringer et al. 2006). More experimentation is needed to determine if chemical cues coupled with visual signals can increase the detection efficiency of PaV1-infected conspecifics by *Panulirus argus.*

Field experiments demonstrated that through redistribution, disease avoidance significantly altered the spatial structure of juvenile lobster populations. Not only did diseased treatment sites have fewer lobsters over time, but the remaining animals moved farther away from the diseased lobster. This indicates that after a diseased lobster finds its way to an aggregation, it is advantageous for neighboring lobsters to quickly move away. These results are consistent with previous experiments that showed urine was diluted to 0.1% of its original strength 1 m from the source (Shabani et al. 2009), and suggests that behaviors driven by the chemoreception of urine probably function over short distances up to 2 m (Derby et al. 2001, Shabani et al. 2009).

The influence of disease on spatial dynamics was correlated with flow, as higher flow regimes demonstrated a lesser effect on distribution patterns. In low flow regimes, the presence of a diseased animal significantly altered the population spatial structure by inducing redistribution away from the infected animal, possibly due to lower turbulence (Finelli 2000). High-velocity flow and physical structures (i.e. shelters in these artificial arrays, or sponges and corals in a natural area) downstream of the odor source may have decreased avoidance behavior on these sites by diluting the odor plume produced by the diseased lobster (Jackson et al. 2007). Reduced chemosensory efficiency correlated to the high flow regime could result from turbulence, reducing the chance of contacting the diseased odor plume (Weissburg & Zimmer-Faust 1993). This possible lack of chemosensory efficiency could have resulted in the lack of significant differences when comparing diseased status within a flow regime, although our results were trending towards significance.

Although there are few studies demonstrating how disease avoidance spatially structures marine populations, presumably due to a lack of evidence for avoidance, there are similarities in terrestrial and freshwater ecosystems. For example, a study on the effects of *Rhopalosiphum padi* virus (RhPV) on the aphid *Rhopalosiphum padi* demonstrated that healthy aphids were attracted to uninfected conspecific aggregations, but not attracted to infected aggregations, spatially segregating infected and healthy individuals on host plants (Ban et al. 2008). Segrega-

tion of diseased animals in any population is apt to take a heavy toll on these individuals, as they have been shown to suffer higher levels of predation, probably due to their lethargic condition (Hudson et al. 1992, Duffy et al. 2005, Behringer & Butler 2010). For example, in the terrestrial environment, predators were found to selectively prey on red grouse *Lagopus lagopus scoticus* with a higher parasite load (of the nematode *Trichostrongylus tenuis*; Hudson et al. 1992). Similarly, in the aquatic environment, Duffy et al. (2005) demonstrated that bluegill *Lepomis macrochirus* selectively feed on *Daphnia dentifera* infected with the bacteria *Spirobacillus cienkowskii*.

Higher levels of predation could result in a population bottleneck for juveniles in shelter-limited habitats when PaV1 prevalence is high (Behringer & Butler 2006, 2010). In habitats that are naturally shelter-limited or shelter-limited due to habitat degradation (Butler et al. 1995, Butler & Herrnkind 1997), the avoidance of infected animals could result in increased predation. As juveniles move away from diseased conspecifics to areas of unknown shelter density or across open substrate, their predation risk is likely to increase, especially among these often small, vulnerable juveniles (Smith & Herrnkind 1992). However, lobsters that do not move away from a diseased conspecific may risk increased mortality from contracting disease. It would seem maladaptive that disease avoidance would cause a predation increase among healthy lobsters, but the overall increase in fitness gained by avoiding disease could be greater.

Clearly, disease can play a pivotal role in animal behavior, and in concert with the physical environment, can have impacts on population spatial structure. The avoidance of disease can influence population dynamics by inducing movement and redistribution, which in turn could affect predation rates. Future studies are needed to empirically test the effect of avoidance on predation and the broad-scale effects of disease avoidance in areas of varying population density and shelter availability. Ecological effects from disease probably drive population dynamics in many other marine host–pathogen systems but are often subtle, requiring observation and experimentation to reveal them.

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