DETECTION OF PAV1-INFECTED CONSPECIFICS AND ITS POPULATION STRUCTURING DYNAMICS IN THE CARIBBEAN SPINY LOBSTER (*PANULIRUS ARGUS*)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my parents

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CL	Carapace length
PaV1	*Panulirus argus* Virus 1
RhPV	*Rhopalosiphum padi* virus
s.d.	Standard deviation

Abstract of Thesis Presented to the Graduate School
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PaV1 (*Panulirus argus* virus 1) is a lethal contact-transmitted pathogen that infects the Caribbean spiny lobster, *Panulirus argus*. Juvenile lobsters are highly susceptible to this pathogen, which prior to mortality causes fundamental changes in their ecology. However, *P. argus* can reduce infection risk by avoiding shelters inhabited by infected lobsters. Based on its role in many other aspects of lobster ecology such as conspecific attraction and mate searching, I hypothesized that olfaction was the most likely mechanism by which lobsters detect PaV1-infected conspecifics. A series of Y-maze experiments were used to test this hypothesis and determine the source of the olfactory cue. Shelter avoidance behavior also has the potential to alter population structuring dynamics, and based on the type of cue, could be affected by local hydrodynamics. To investigate this, shelters and diseased cues were manipulated for wild populations in both high and low flow environments. Results showed that diseased avoidance is driven by olfaction via urine release, and moreover, the olfactory cue alone was equivalent in effectiveness to having a diseased lobster present and visible. When given a choice between sheltering with diseased or non-diseased conspecifics, lobsters rarely sheltered with diseased individuals. In the field, detection of PaV1-infected conspecifics can alter structuring dynamics

of natural populations especially under low flow regimes by inducing emigration and redistribution of non-diseased animals relative to diseased conspecifics. In shelter-limited environments, as occurs in many regions of the Caribbean, the unavailability of shelters due to disease avoidance could increase juvenile mortality and potentially impact future adult stocks.

CHAPTER 1
INTRODUCTION

Olfaction is a sense widely used by organisms to understand their environment (Ache and Young 2005). The efficiency of olfactory-mediated interactions is relative to the transmission and range of chemical detection, which in the terrestrial environment is limited by chemical volatility (Liley 1982) and bioenergetic cost of chemical production (Wilson 1975). In terrestrial organisms, olfaction is vital for reproduction (Averhoff and Richardson 1974, Carter et al. 1980), predation-prey interactions (Apfelbach et al. 2005, Rosell and Sanda 2006), and interactions between organisms (Hurst 1990, Moore 1997, Bonadonna and Nevitt 2004). Compared to aquatic olfaction, terrestrial olfaction is well understood, with hundreds of chemical signals described for terrestrial arthropods (Herberholz 2007).

Although better understood in the terrestrial environment, olfaction can dominate the sensory realm in the aquatic environment (Ache and Young 2005). In aquatic environments, the efficiency and effectiveness of chemical transmission is determined by the ability of a chemical to dissolve (solubility) and therefore disperse within its environment (Liley 1982). Chemicals can be released from numerous anatomical sources, such as the slime covering of fish (Wilson 1975, Liley 1982), gill membranes (Liley 1982), and through urine (Bamber and Naylor 1997). Aquatic organisms employ olfaction to find food (Carr and Derby 1986, Finelli et al. 2000), migration/homing (Hasler and Wisby 1951, Herrnkind 1969, Barbin et al. 1998), predator avoidance (Liley 1982, McCormick and Larson 2007), and intraspecific communication (Ameyaw-Akumfi and Hazlett 1975, Stacey 2003, Diaz and Theil 2004). For example, food searching in the California spiny lobster, *Panulirus interruptus,* is driven by the chemoreception of adenosine triphosphate (ATP) in the water column (Zimmer-Faust 1993). Likewise, homing by the black rockfish, *Sebastes inermis,* observed by their strong attraction to their habitat after

displacement, is accomplished through olfactory pathways (Mitamura et al. 2005). Olfactory mediated predator avoidance is observed in the goby, *Asterropteryx semipunctatus,* which can learn and associate novel chemical signals with danger (Larson and McCormick 2005). Olfaction can also be found in mate searching by the female rock shrimp, *Rhynchocinetes typus,* which is mediated predominantly through olfaction with females initially attracted to chemical signals emitted by males (Diaz and Theil 2004). Chemical communication is used by many decapods for a wide range of social interactions (Herberholz 2007).

Among decapods, the source of the olfactory cue is often found in the urine (Atema 1986, Bushmann 1999, Breithaupt and Eger 2002) that contains pheromones used to mediate conspecific interactions (Wilson 1965, Bamber and Naylor 1997, Horner et al. 2008). Common urine-mediated intraspecific interactions among decapods include: aggregation and homing (Childress and Herrnkind 1997, Nevitt et al. 2000, Bushmann and Atema 1997), social interactions (Zimmer-Faust et al. 1985, Bushmann 1999, Shabani et al. 2009), individual recognition (Karavanich and Atema 1998 a,b), reproduction (Atema and Engstrom 1971, Bamber and Naylor 1997, Kamio et al. 2008), and possibly disease detection (Behringer et al. 2006). The widespread use of urine in common interactions is credited to its rich soluble protein content, which increases its dispersal, and the ability of decapods to use appendage-generated currents (Caldwell 1992, Gleeson et al. 1993, Kamio et al. 2008). Therefore it's plausible that, urine could be employed in disease detection. Moreover its metabolic waste concentration can be used to assess the internal and overall health of an organism (Breithaupt and Eger 2002, Atema and Steinbach 2007). Like individual recognition in many crustaceans, (Karavanich and Atema 1998 a,b, Hemsworth et al. 2007) viral detection could lead to an olfactory imprint of disease causing altered behaviors (Zulandt Schneider et al. 2001).

Crustaceans detect chemical signals in the aquatic environment through chemoreceptors (Laverack 1988, Cate and Derby 2002) located primarily on the maxillipeds, pereiopods, antennules, and antennae; however, crustaceans can have chemosensory cells on almost any part of the body (Atema and Steinbach 2007, Herberholz 2007). Maxillipeds, pereiopods, and other portions of the body are used for food tasting through tactile processes (Atema and Steinbach 2007). The antennules, considered the main sensory organs in many crustaceans, have the largest proportion of chemosensory cells and are used for assessing their environment (Horner et al. 2008). When detecting odor plumes the antennules are commonly flicked to create flow that disrupts the chemical boundary layer around the sensory cells, allowing a new signal to be processed (Schmitt and Ache 1979, Goldman and Patek 2002, Daniel et al. 2008).

Olfaction can also be a population structuring mechanism, used by many crustaceans to form conspecific aggregations (Bushmann and Atema 1997, Childress and Herrnkind 1997, Ritz 2000). Conspecific aggregations are important because they decrease predation rates (Zimmer-Faust and Spanier 1987, O'Brien and Rtiz 1988, Smith and Herrnkind 1992), provide reproductive refuge (Bushmann and Atema 1997, Atema and Steinbach 2007), and reduce energetic costs in swimming crustaceans (Ritz 2000). For example, the spiny lobster, *Panulirus interruptus*, uses olfaction to sense conspecific olfactory cues when searching for shelter aggregations (Zimmer-Faust et al. 1985). Likewise, the American lobster, *Homarus americanus*, will form shelter aggregations in which the male protects the newly molted female during reproduction (Bushmann and Atema 1997). Many factors influence the formation of shelter aggregations including the number of suitable shelters, conspecific density, and predation rates (Zimmer-Faust et al. 1985, Nevitt et al. 2000, Behringer and Butler 2006).

While chemosensory cues are important in social interactions, visual cues have also evolved in organisms that inhabit shallow, well-lit environments because they can be exchanged rapidly and efficiently from a distance (George and Main 1966, Acquistapace et al. 2002, Baldwin and Johnsen 2009). Visual communication is used in intraspecific interactions as found in female Lake Victoria cichlids, *Haplochromis nyererei* complex, whose mate choice depends on coloration and display rates of suitable males (Seehausen and van Alphen 1998). Likewise, Baldwin and Johnsen (2009) found that mate choice of the blue crab *Callinectes sapidus*, can be stimulated solely by visual cues with males being attracted to females with red dactyls. Another example of the importance of visual cues lies in the diurnal migration of spiny lobsters wherein they align themselves in a straight formation via visual cues (Herrnkind 1969).

Combined visual and chemical signals (signal enhancement) are also commonly required in nature to induce behavioral responses because they increase the efficiency of signal transmission (Smith 1969, Acquistapace et al. 2002, Herberholz 2007). Bimodal communication is employed in intraspecific interactions such as the male European freshwater crayfish, *Austropotamobius pallipes* (Acquistapace et al. 2002), and in the male snapping shrimp, *Alpheus heterochaelis* (Hughes 1996). The female southern rock lobster, *Jasus edwardsii*, uses a combination of tactile, olfactory, and visual senses to distinguish between potential reproductive partners (Raethke et al. 2004). Signal enhancement plays a role in intraspecific interactions but also could play a vital role in forming shelter aggregations and population structuring due to its widespread use.

Regardless of the cue driving aggregation or population structure, it was long assumed that increased disease prevalence was a cost of sociality because social interactions can increase the spread of contact-transmitted disease (Freeland 1979, Wright and Gompper 2005).

However, we now know that might not necessarily be true. The ability to detect and avoid diseased conspecifics can reduce disease transmission by affecting population structure (Behringer et al. 2006, Ban et al. 2008). Although it has been shown that disease avoidance can affect the population structure of terrestrial and aquatic organisms (Thompson et al. 2002, Ban et al. 2008), only anecdotal data exists of these effects in the marine environment (Sousa 1991, Thompson et al. 2005, Behringer et al. 2006). For instance in the aquatic realm, the western mosquitofish, *Gambusa affinis* (Tobler and Schlupp 2008), and the aphid, *Rhopalosiphum padi* (Ban et al. 2008), avoid infected conspecifics creating segregation within the population. In the marine realm, in which crustaceans shelter gregariously, the avoidance of shelters inhabited by infected conspecifics (Behringer et al. 2006) effectively removes the use of that shelter from those available for habitation, which in turn could have population spatial structuring affects if local disease prevalence is high.

In marine environments with variable flow velocity, the efficiency of olfaction can be altered (Kleerekoper 1967, Weissburg and Zimmer-Faust 1993, Finelli 2000). For instance, Finelli (2000) demonstrated that as flow velocity increases, odor plume concentration decreases and physical structures (e.g., sponges, octocoral, seagrass) downstream of the odor source turn the single odor plume into many small, dilute plumes. Another example is found in many teleost fish which cannot locate the source of stagnant olfactory cues, but can locate odor source when odor plumes are coupled with water flow (Kleerekoper 1967). Conversely, the efficiency of olfaction in blue crabs in high flow environments is decreased due to increased turbulence around the antennule boundary layer (Weissburg and Zimmer-Faust 1993). Furthermore, if disease detection occurs via olfaction, local hydrodynamics could influence the area of avoidance.

The Caribbean spiny lobster, *Panulirus argus* (Latrielle 1804), is one example of a decapod that employs visual and olfactory signals in an environment with variable flow. This gregarious animal is attracted to shelters inhabited by conspecifics by using an aggregation odor. This subsequently decreases predation by enabling conspecifics to find suitable shelter three times faster (Zimmer-Faust et al. 1985, Childress and Herrnkind 1997, Childress and Herrnkind 2001). This olfactory-driven behavior can help decrease predation on these vulnerable animals which are preyed upon by a suite of predators (Smith and Herrnkind 1992, Mintz et al. 1994, Schratwieser 1999). Other daily intraspecific interactions are also commonly mediated by olfaction (feeding - Derby and Cate 2001, predator avoidance - Berger and Butler 2001, aggressive behaviors - Shabani et al. 2009) and visual cues (migration - Herrnkind 1969).

Their social nature implies that they should be highly susceptible to contact transmitted diseases, but Behringer et al. (2006) demonstrated that healthy *P. argus* can detect and avoid conspecifics infected with the lethal virus *Panulirus argus* virus 1 (PaV1). However, infected individuals remain attracted to other conspecifics (non-diseased or diseased) and will actively try to aggregate with them (Behringer et al. 2006). PaV1 is a contact transmitted pathogen that prior to mortality causes fundamental changes in lobsters (Shields and Behringer 2004, Butler et al. 2008). Such fundamental changes include lethargy, tissue ischemia, depletion of energy reserves, and mortality (Shields and Behringer 2004). Although PaV1 can infect lobsters of all sizes, juveniles are most susceptible to this pathogen, succumbing more quickly than adults (mortality within 90 days) (Shields and Behringer 2004, Butler et al. 2008). Although it is known that lobsters can detect PaV1-infected conspecifics, the mode of detection, source of the cue, and effects on population structuring dynamics are unknown. Possible implications of disease avoidance in a shelter-limited habitat could be emigration and redistribution away from

infected conspecifics. Using the Caribbean spiny lobster as a model species, I tested the implications of disease avoidance on juvenile population structuring dynamics.

Hypotheses: Determine the primary sense (visual versus olfactory) used in detecting PaV1-infected lobsters, and the source of the olfactory cue.

Ho_1: Caribbean spiny lobsters do not use chemical or visual cues to detect PaV1-infected conspecifics

H_{A1}: Detection of PaV1-infected spiny lobsters by visibly healthy lobsters occurs via chemical and visual signals.

Ho_2: If olfaction is critical to detecting conspecific cues relative to PaV1 in spiny lobsters, urine does not play a factor

H_{A2}: If olfaction is critical to detecting conspecific cues relative to PaV1 in spiny lobsters, urine is the source of the olfactory cue.

Determine the range of infected lobster avoidance by non-infected lobsters.

Ho_1: PaV1-infected lobster avoidance non-infected lobster display, is not associated with distance from cue source

H_{A1}: PaV1-infected lobster avoidance non-infected lobster display, is associated with the distance from the cue source.

Evaluate how PaV1 affects juvenile population structure in the wild.

Ho_1: PaV1 avoidance does not alter the spatial structure of wild spiny lobster populations

H_{A1}: PaV1 avoidance alters the spatial structure of wild spiny lobster populations.

Evaluate the effect of flow on the spatial structuring function of the PaV1 avoidance behavior.

Ho_1: Spatial avoidance of cues from PaV1 diseased lobster by non-diseased lobsters is not positively associated with water flow rate

H_{A1}: Spatial avoidance of cues from PaV1 diseased lobster by non-diseased lobsters is positively associated with water flow rate.

CHAPTER 2
METHODS

Mode of PaV1-infected Conspecific Detection and Cue Source

Experimental Lobsters

During the summer of 2010, juvenile Caribbean spiny lobsters, 25-50 mm carapace length (CL), were hand-collected from hard-bottom habitat (< 3 m depth) in Florida Bay, Florida (Figure 2-1). Lobsters were held in UV-treated flow-through tanks under natural photoperiod. Lobsters were fed shrimp *ad libitum* every other day. Of these lobsters, 30 were inoculated with PaV1 by injecting 0.1 mL of hemolymph from a visually PaV1-infected lobster (Shields and Behringer 2004) through the membrane between the basis and the ischium of the fifth walking leg (Behringer et al. 2008). Inoculated animals were held separately from non-diseased animals in recirculating tanks to ensure they were in a similar infection state. This inoculation method typically produces a 95% infection rate in a laboratory setting (Butler et al. 2008).

Experimental Setup

To determine the mode of PaV1 detection, Y-maze experiments (Rebach 1996, Ratchford and Eggleston 1998, Diaz and Thiel 2004) were conducted in the summer-fall 2010. Four 94cm long x 62cm wide x 20cm tall Y-mazes (79 L) with a 72cm long x 18cm tall central divider were constructed out of 1.3cm plywood coated with waterproof epoxy (Figure 2-2). To attract lobsters, each upstream arm of the Y-maze held a conditioned artificial shelter (2 stacked concrete bricks; 20cm x 10cm x 5cm) (Butler and Herrnkind 1997, Behringer and Butler 2006). Through the center of the shelters, unfiltered, ambient seawater (25 ± 1ºC and 35 ± 1 ppt) was gravity fed from isolated 121 L head tanks at a rate of 4.00 mL/s. From the shelters, water flowed downstream at a rate of 1 cm/s to an 18cm tall, 1.9cm diameter stand pipe. Dye tests

confirmed that water from each Y-maze arm was unidirectional and restricted to its respective side before converging at the base of the Y-maze.

Chemical Detection of PaV1-infected Conspecifics

All Y-maze experiments were conducted under natural photoperiod for an experimental duration of 8h during the lobsters' most active state, ending 1h post-dawn (when they are actively searching for shelters). In all olfactory Y-maze trials, 2 lobsters (specific treatments discussed below) were placed in a randomly assigned head tank 4h prior to the start of the trial to infuse the water with their chemical signal. At the onset of the trial, a non-diseased (visually assured - Shields and Behringer 2004) lobster was measured (CL; to the nearest 0.1 mm), sexed, and positioned at the base of the Y-maze behind a porous screen. Following a 5min period to reduce handling induced stress on the focal animal, the screen was removed and the lobster was allowed to move about freely for the duration of the trial. Preliminary observations indicated that lobsters (n = 12) sampled and thus received cues from both branches of the Y-maze. Eight hours into each trial, the shelter and associated treatment that the focal lobster selected were recorded. Between trials the setup was drained, flushed with fresh water three times (Diaz and Thiel 2004), and allowed to air dry for 10h.

To determine if *P. argus* can chemically detect and avoid PaV1-infected conspecifics, 3 olfactory experiments were conducted. The first experiment tested the olfactory cues emitted from non-diseased lobsters in one head tank versus olfactory cues emitted from seawater in the second head tank (n = 30). This experiment provided a baseline on the sheltering behaviors of focal animals and was expected to depict the gregarious nature of non-diseased conspecifics. The second experiment tested the olfactory cues emitted from diseased lobsters in one head tank were tested against olfactory cues emitted from seawater in the second head tank (n = 20). In contrast to the previous experiment, this experiment provided information on the avoidance of

PaV1 infected individuals. Finally, to determine if lobsters favored non-diseased conspecifics over diseased conspecifics, olfactory cues emitted from non-diseased lobsters in one head tank were tested against the olfactory cues emitted from a head tank that held diseased lobsters (n = 21). This experiment coupled the natural gregarity of focal animals to non-diseased conspecifics with their natural avoidance of diseased conspecifics to determine their sheltering choice. In combination, these experiments provided information as to whether lobsters merely avoided diseased lobsters or rather were attracted to non-diseased conspecifics. Crustaceans do not typically demonstrate a side preference (Rebach 1996) or directional bias (Zimmer-Faust 1993) during Y-maze experiments, so these factors were ignored.

PaV1-infected Conspecific Urine Detection

To specifically test whether PaV1-infected conspecifics were detected chemically through urine, Y-maze experiments were conducted (n = 30) with the nephropores of diseased lobsters blocked (Bushmann 1999, Zulandt Schneider et al. 2001). The nephropores of the PaV1 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 the complete coverage of the gel plug. To reduce experimental bias, non-diseased control lobsters were removed from their holding tank, blotted dry (no glue applied) and held out of water for the same time period as treatment lobsters. Non-diseased animals did not have their nephropores blocked because the purpose of the experiment was to see if blocking the nephropores of diseased animals in effect made them similar to non-diseased animals. Observations of the gel plug illustrated that it could be removed post-experiment without detriment to the lobster. After application of the gel plug, treatment lobsters (nephropore-blocked diseased blocked) were placed in one head tank while non-diseased lobsters (unblocked) were placed in the other head tank 4h prior to each trial. If the PaV1 avoidance cue occurred as a consequence of urine emitted

from the nephropores, then the blocked nephropore lobsters would expected to be chemically 'invisible' to the focal lobsters in this experiment. The preceding Y-maze experiments were used as controls because they served as a baseline of lobster sheltering behavior, providing focal lobster choice on shelters emitting olfactory cues of diseased or non-diseased animals.

Visual Cues in PaV1 Detection

To determine if lobsters could visually detect PaV1-infected conspecifics, Y-mazes were modified by installing a sheet of glass (18cm tall x 28cm wide) 6cm in front of the each upstream section of the Y-maze (Figure 2-3) secured with silicone caulking to ensure that water was not exchanged between the Y-maze and the partitioned section. Within this partitioned section two bricks (20cm x 10cm x 5cm) standing on end were placed to either side of the partition to keep the treatment lobster in the middle of the Y-maze branch and in view of the focal lobster. The procedure was the same as described above except that no lobsters were present in the head tanks and the water was released downstream of the glass partition. This experiment included three treatments: non-diseased lobster visual cues versus seawater only (n = 30), disease lobster visual cues versus seawater only (n = 23), and non-diseased lobster visual cues versus diseased lobster visual cues (n = 30).

Data Analyses

To determine if the sheltering choice deviated from random, a two-tailed binomial with the probability of sheltering set at 50% (random sheltering) was used. The treatments were considered independent and the sheltering probability stayed the same throughout the experiment (McClave and Sincich 2009). The power of the test (1- β) indicated the probability that the test would correctly reject the null hypothesis (sheltering probability = 0.5). The value of the type II error (β) decreased as the observed results (probability) deviated from the null hypotheses (0.5)

(McClave and Sincich 2009). Significance was determined at $\alpha = 0.05$ for this and all other experiments as described below.

PaV1-infected Conspecific Avoidance Range

Experimental Setup and Procedure

To determine the range at which PaV1-infected conspecifics elicits an avoidance reaction, a flume tank experiment was conducted. Previous studies with American lobster, *Homarus americanus,* indicated that they lose olfactory memory of previously encountered individuals after being separated for 2wks (Karavanich and Atema 1998 a,b). Similar observations have been observed in the common crayfish, *Cherax destructor* (Hemsworth et al. 2007), and rusty crayfish, *Orconectes rusticus* (Zulandt Schneider et al. 2001). Accordingly, diseased lobsters in the present study were isolated from non-diseased lobsters for 4wks prior to the experiment to eliminate the chance of any olfactory recognition.

Trials were conducted at night in a flume tank with a flow rate of 1.34 cm/s. This flow rate was used because, based on the flow velocity at field sites, it was a flow rate that would not create excessive turbulence that would disrupt the odor plume. The bottom of the tank was divided into 3 quadrants (25cm long x 28cm wide) (Figure 2-4). At the downstream end of the experimental arena a porous screen was placed to keep lobsters from sheltering behind the standpipe. The upstream end of the experimental arena contained a brick shelter used to attract the focal lobsters to that end of the flume. Water was delivered to the shelter end of the tank via a 15cm long PVC manifold. This manifold created a unidirectional flow and prevented focal lobsters from going beyond the shelter.

To start the trial a treatment lobster (non-diseased n = 19; diseased n = 29) was tethered to the center of the artificial shelter using a monofilament harness secured to the carapace with cyanoacrylate gel (Krazy Glue®). A metal swivel connected between the carapace and tethering

line allowed the lobster to move about freely within the artificial shelter but prevented their movement into the experimental arena. A size-matched non-diseased lobster (± 5 mm CL of tethered animal) was then positioned at the downstream end of the tank for 5min behind a porous screen to reduce handling induced stress, after which the focal lobster was allowed to move about freely for 1h. Diseased treatment animals were used in multiple trials due to the lack of diseased animals. Trials were recorded with a digital video camera under a red light for subsequent analysis. Red lighting falls at the far end of the maximum spectral sensitivity of crustaceans (> 700nm wavelength) therefore minimizing any visual stimuli that might alter focal animal behavior (Cronin 1986). Between each trial the flume was drained, cleaned, and flushed with freshwater for 1h.

Data Analysis

Video of the trials was used to determine the amount of time spent sheltered or within each quadrant (shelter, 0-50 cm, 50-100 cm, and 100-150 cm). The mean amount of time spent in each quadrant was compared using an ANOVA with distance (sheltered, 0-50 cm, 50-100 cm, and 100-150 cm) and treatment (non-diseased or diseased) as factors. ANOVA assumptions were assessed by comparing standardized residual plots (residuals divided by the data standard deviation; s.d.) for this and other experiments as described below.

Influence of PaV1 Avoidance on Juvenile Population Structuring Dynamics

Experimental Setup

In fall 2010, a tagging experiment was conducted in Florida Bay (USA) to assess the effects of PaV1-infected conspecific avoidance on spatial population structuring dynamics (Figure 2-1). The tagging experiment was conducted in artificial shelter arrays designed to take into account olfactory capabilities of *P. argus* by having multiple distances from the cue source (discussed below). Such capabilities include exchanging social signals over short distances, < 60

cm (Shabani et al. 2009), and orienting to odor plumes 2m away (Derby et al. 2001). Eight artificial shelter arrays (Figure 2-5), were placed 100m apart in hard-bottom habitat (~2m depth) typically occupied by juvenile lobsters (Herrnkind et al. 1997). Only large areas (at least $4m^2$) devoid of natural shelters were selected as sites for array placement. Artificial shelters were double stacked concrete blocks (40cm x 20cm x 10cm) conditioned for 3mo. in the ocean prior to use (Behringer and Butler 2006). Although the main focus of the study was on population structuring associated with PaV1-infected conspecific avoidance, I also sought to determine the importance of flow rate on and structuring effect observed. Therefore, 4 arrays were placed in low flow environments and 4 arrays placed in a high flow environments. An average flow (cm/s) (n = 3 for each flow regime) for the differing regimes was calculated using a digital/mechanical flow meter (G.O. Environmental, Model 2030) set at the site for 24h to record mean daily flow for each site and a t-test was employed to determine significant differences between the flow regimes.

Experimental Procedure

After colonization of the shelters (at least 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 their CL, shelter location, mark it with a unique color-coded antenna tag, and return it to its respective shelter. After the initial surveys (t_0), a visibly non-diseased or diseased juvenile collected from nearby was tethered as described above with a 20cm piece of monofilament line to the central block in the shelter array. Subsequent surveys were conducted at 24h (t_{24}) and 48h (t_{48}) to allow untethered lobsters time to redistribute in response to the tethered treatment. During the t_{24} and t_{48} surveys, 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 antennae tag was observed without actually capturing the lobster to reduce stress-

induced movement. To understand the effects of stress caused by divers on lobster sheltering behavior, an initial survey (n = 6) was conducted 24h before t_0 surveys. This pre-trial survey used the same methods described above except no animal was tethered to the central shelter after the survey. This provided information on the amount of emigration from the site caused by the tagging process.

Data Analysis

The geometric formation of the shelters allowed multiple distances to be calculated from the central stimulus (Berger and Butler 2001). Three measurements were taken from this formation: (1) lobsters 0.5m from central shelter, (2) lobsters 1m from the central shelter, and (3) lobsters 2m from the central shelter. Two separate repeated-measures ANOVAs were used to reduce the variability associated with time with the response variable log-transformed. Optimum power transformation was found by using a Box-Cox transformation within SAS that finds the power transformation that maximizes reduction in model variability. Separate analyses were performed due to unequal time factors between the two respective response variables; the first analysis (response = count) had three entries per site (t_0, t_{24}, t_{48}) while the second (response = distance moved) had two data points per site (between t_0 - t_{24} and between t_{24} - t_{48}). Conclusions from both analyses were taken from the Type III tests of fixed effects. Significant ANOVAs were followed up with Tukey-Kramer multiple comparison tests to determine the source of differences (McClave and Sincich 2009). Multiple comparisons tests compared marginal means which are means where the sum of square errors is minimized in the overall solution, decreasing variability between the comparisons (McClave and Sincich 2009).

The second analysis was used to determine if the movement of lobsters changed over time (redistribution) when a treatment animal was present. Net movement was determined by adding the movement towards the treatment and subtracting movement away from the treatment

of each individual lobster on the site (lateral movement = 0). Emigration and immigration was subtracted or added to the total, respectively, relative to the abandoned/inhabited shelter distance from the central treatment. Immigration to shelters closest to the central treatment (0.5m) was scored as +2.5 because the immigrating lobster had to traverse closer to the central shelter. Immigration to shelters 1m from the central treatment was scored a +2. Likewise, immigration to shelters 2m from the central treatment was scored a +1. Emigration was scored inversely to immigration; i.e., if the abandoned shelter was 0.5m from the central treatment than it was scored a -2.5.

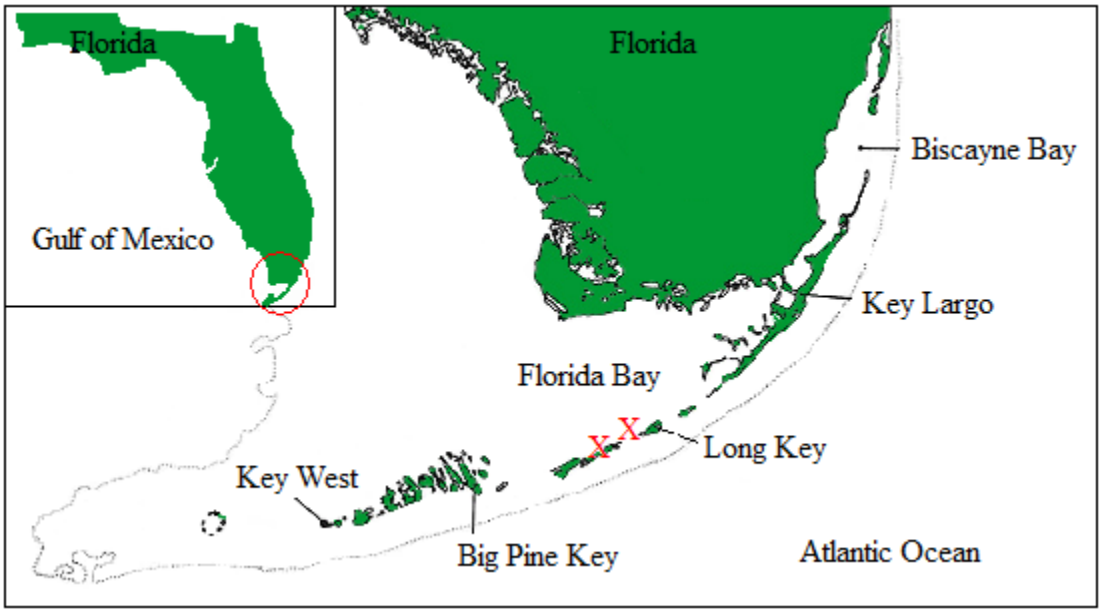

Figure 2-1. Florida Bay, FL (USA), the location of lobster collection and study sites. Area of study sites are denoted with an "X" (4 sites at each X) (Image credit: Florida Museum of Natural History).

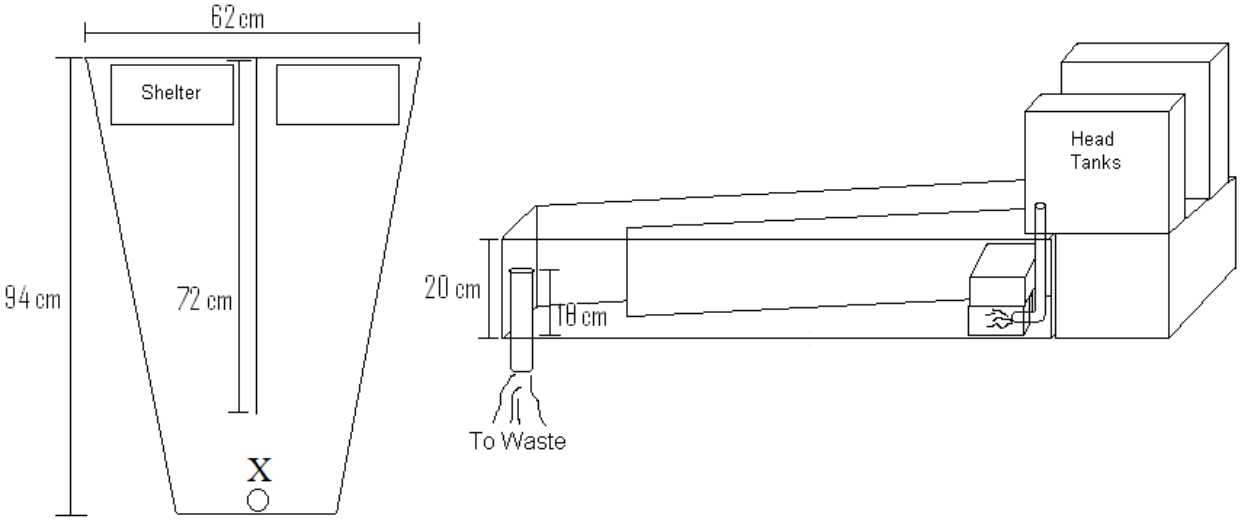

Figure 2-2. Y-maze design used to determine the mode of PaV1-infected conspecific detection and the source of the cue. "X" denotes the placement of focal lobster at the start of trials.

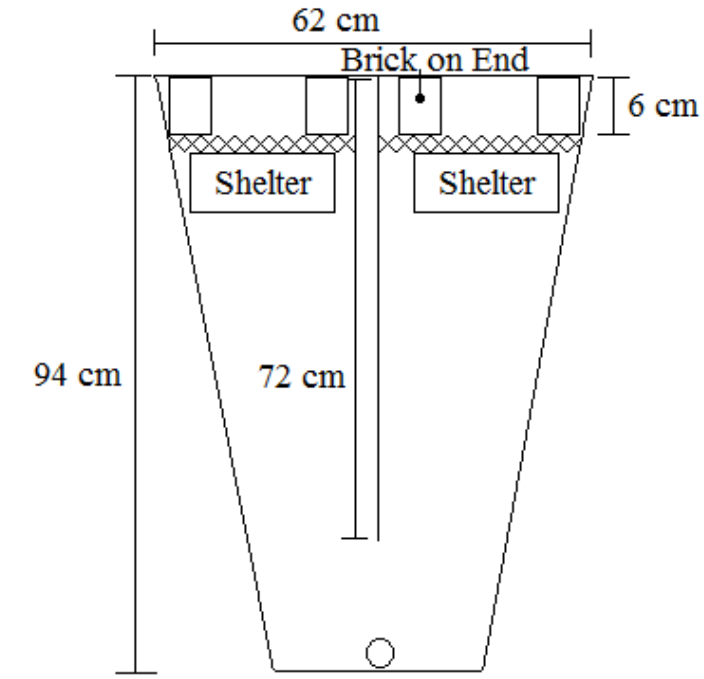

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Figure 2-3. Y-maze set up for experiments used to determine if detection of PaV1-infected conspecifics occurs visually. Hatch-marked section represents a glass partition.

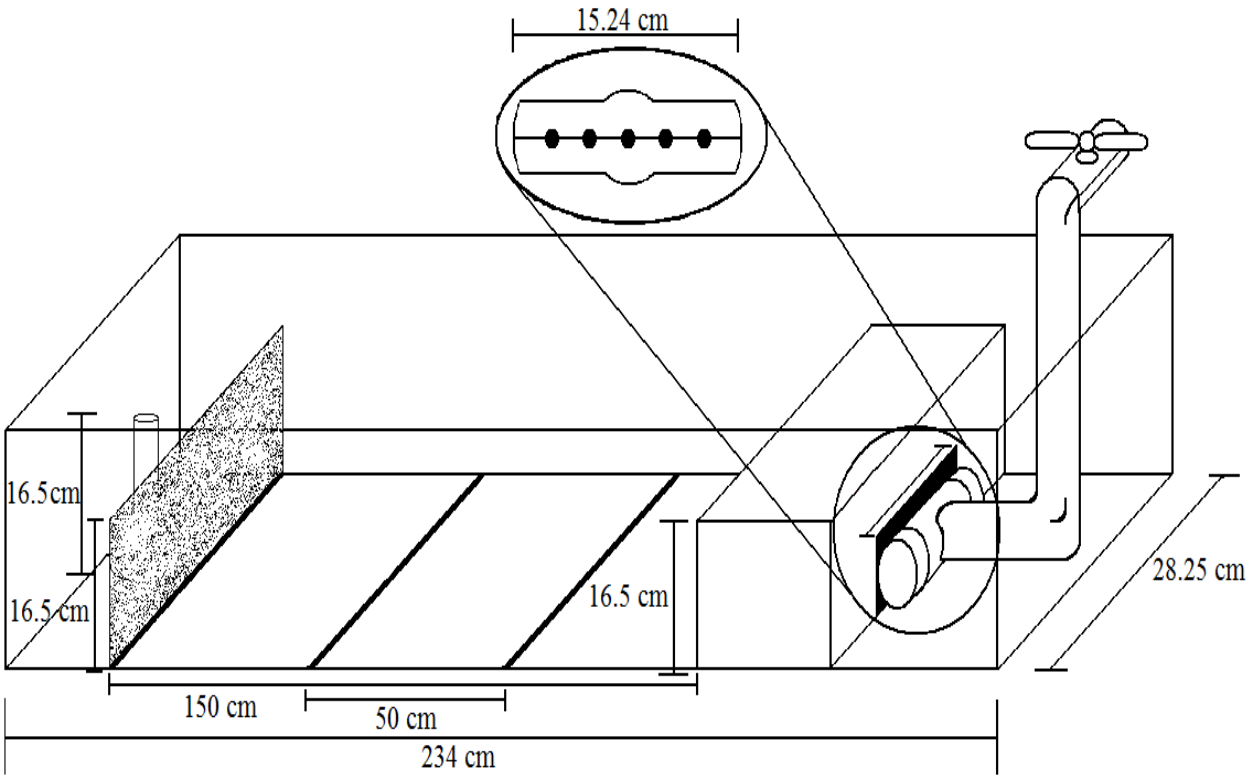

Figure 2-4. Flume and quadrants used in the determining of the range of PaV1 avoidance.

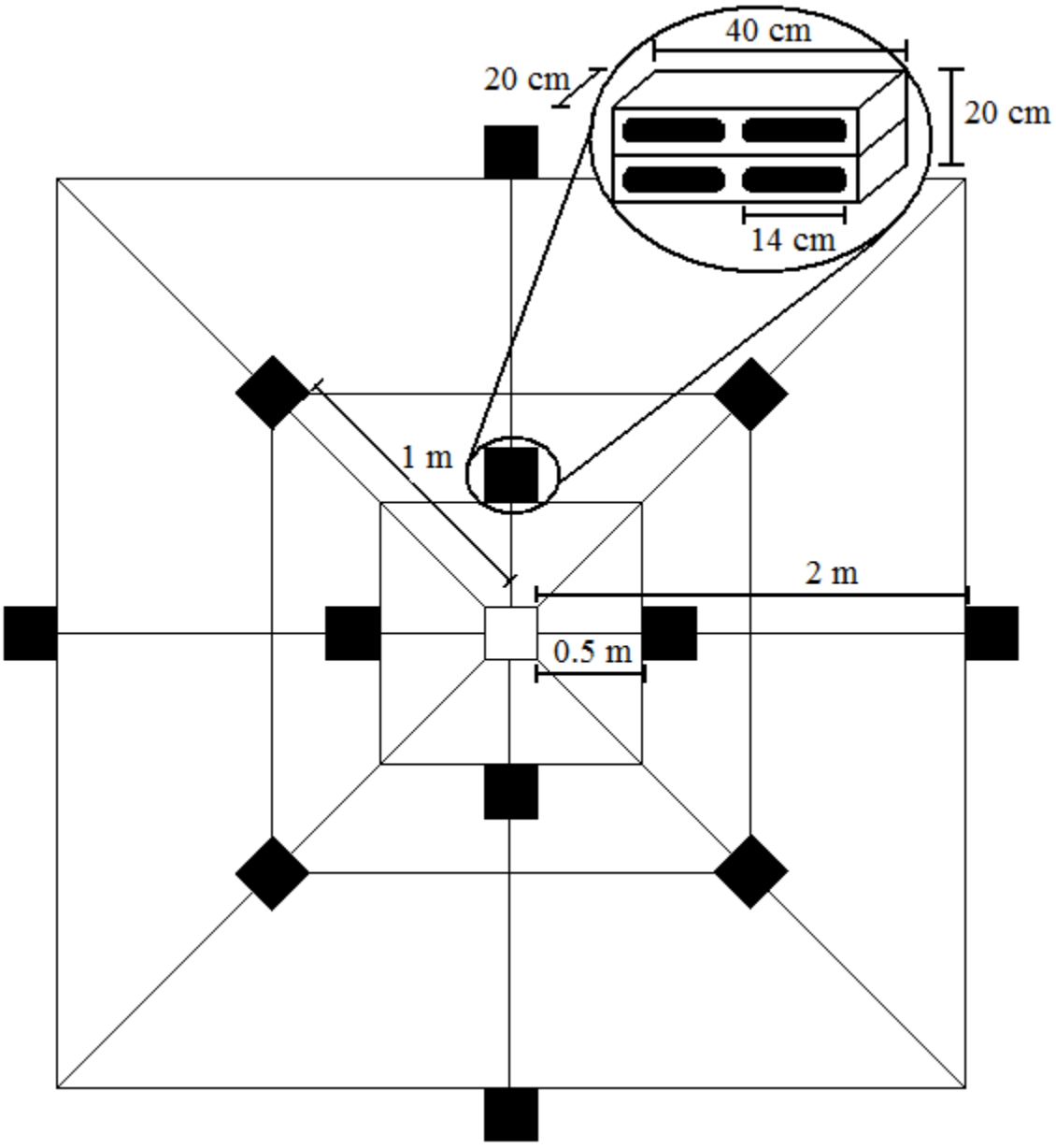

Figure 2-5. Geometric formation of artificial shelters used to determine the population structuring influence of PaV1-infected conspecific avoidance.

CHAPTER 3
RESULTS

Mode of PaV1-infected Conspecific Detection and Cue Source

Non-diseased treatment animals in the Y-maze experiments ranged in size from 25.0 - 49.0 mm CL with a mean of 34.1 ± 6.3 mm CL s.d. and diseased treatment animals ranged in size from 22.3 - 49.0 mm CL with a mean of 31.3 ± 5.1 mm CL s.d. Non-diseased treatment animals were significantly larger than diseased treatment animals (t-test: df = 412.41; t-value = 4.98; $P < 0.0001$) but because diseased animals were significantly smaller (less urine output of smaller individuals; Shabani et al. 2009) any bias would have been in favor of not finding a difference between disease and non-diseased control treatments. Focal lobsters ranged in size from 22.1 - 49.8 mm CL with a mean of 33.4 ± 6.3 mm CL s.d. Results of the olfactory cue experiments are summarized in Table 3-1. Focal animals in the non-diseased versus seawater only treatment selected the shelter with non-diseased olfactory cue 67% of the time, but did not differ significantly from random sheltering ($P = 0.0987$; $1-\beta = 0.432$) (Figure 3-1). Focal lobsters in the diseased versus seawater only treatment preferred to shelter with no conspecific odor over diseased odor with sheltering probability significantly different than random ($P = 0.0414$; $1-\beta = 0.617$). Focal lobsters in the non-diseased versus diseased treatments selected shelters with non-diseased olfactory cues 86% of the time ($P = 0.0015$; $1-\beta = 0.932$). When the nephropores of diseased lobsters were blocked, focal lobsters selected shelters supplied with water from non-diseased or diseased head tanks equally ($P = 1.0000$; $1-\beta = 0.043$). In no case was sheltering affected by treatments in visual cue experiments (Table 3-1).

PaV1-infected Conspecific Avoidance Range

ANOVA assumption checking indicated that standardized residuals were independent of one another and had approximately equal variances. Although standardized residuals did not

conform to a normal distributed (Shapiro-Wilk normality test: $P < 0.0001$), the data was considered normally distributed due to the general robustness of ANOVAs in determining significant differences in means even if the data are not normally distributed.

During the flume tank experiment to determine the range of PaV1-infected conspecifics by focal lobsters, treatment lobsters ranged in size from 25.1 - 39.2 mm CL with a mean of 32.2 ± 4.1 mm CL s.d. Focal lobsters ranged in size from 23.6 - 38.1 mm CL with a mean of 30.4 ± 3.7 mm CL s.d. Results indicate that the presence of a diseased or non-diseased animal alone was not significant ($P = 0.9986$), but distance from disease presence ($P < 0.0001$) and interaction between distance and disease presence differed significantly ($P = 0.0002$) (Table 3-2). The distances 0 - 50 cm ($P = 0.2393$) and 50 - 100 cm ($P = 0.6351$) did not differ significantly between treatments, but 100 - 150 cm ($P = 0.0352$) and within shelter ($P = 0.0002$) differed significantly (Figure 3-2).

Influence of PaV1 Avoidance on Juvenile Population Structuring Dynamics

ANOVA assumptions checking indicated that all assumptions were met, the standardized residuals were found to be normally distributed, independent of one another, and had approximately equal variances. A Shapiro-Wilk normality test among the standardized residuals indicated that they conformed to a normal distribution (count analysis: $P = 0.7780$; movement analysis: $P = 0.1686$).

In 28 experimental trials (disease n = 12, non-disease n = 16 or high flow n = 14, low flow n = 14) 258 natural focal lobsters were caught and tagged, ranging in size from 10.0 - 65.1 mm CL with a mean of 29.6 ± 7.5 mm CL s.d. There was a mean of 8.1 ± 4.8 wild lobsters per site at t_0 surveys. In the16 non-diseased (control) experiments, tethered lobsters ranged in size from 29.5 - 49.8 mm CL with a mean of 37.0 ± 6.4 mm CL s.d., and in the 12 disease experiments tethered lobsters ranged in size from 24.3 - 37.1 mm CL with a mean of 29.9 ± 3.9

mm CL s.d. Carapace length of tethered animals differed significantly between treatments (t-test: df = 25.25; t-value = -3.01; P = 0.0058), but because diseased animals were significantly smaller (less urine output of smaller individuals; Shabani et al. 2009) any bias would have been in favor of not finding a difference between disease and non-diseased control treatments. Flow at low flow sites (0.20 cm/s; ± 0.74 s.d.) was significantly less (df = 2.02; t value = 4.79; P = 0.0403) than flow at high flow sites (7.94 cm/s; ± 2.79 s.d.). Repeated-measures ANOVA analysis indicated no significant difference between pretrial and t_0 wild lobster counts on the sites (df = 9; F = 0.00; P = 0.9665), nor were numbers affected by flow regime (df = 22; F = 0.04; P = 0.8509), or distance (df = 22; F = 0.08; P = 0.9278).

Trial surveys comparing the changes in lobster count over time indicated that health status (P = 0.0009) and time (P < 0.0001) differed significantly (Table 3-3) (Figure 3-4). Multiple comparison analysis of the marginal means was used to determine that within the diseased treatment the number of lobsters at low flow sites was not significantly different than the number of lobsters at the high flow sites (P = 0.0810). Within the low flow treatment, there were significantly fewer lobsters present at diseased sites (P = 0.0010). Non-diseased treatment sites did not differ significantly between flow rates (P = 0.9989) nor did health status differ significantly under high flow rates (P = 0.7408) (Table 3-4).

Within health status, the number of lobsters located within shelters at different distances from the central shelter also differed significantly. The number of lobsters at 0.5m from the central shelter differed significantly between health status (P = 0.0314) while the number of lobsters at 1m (P = 0.0690) and 2m (P = 0.1929) from the central shelter did not differ significantly (Table 3-5). At the distance of 0.5m there were fewer lobsters located at the diseased sites compared to the non-diseased sites. Within the diseased or non-diseased

treatments there were no significant differences between the distances (Table 3-5). Multiple comparison analysis of the interaction of heath status, flow rate, and distance from central shelter revealed that at a distance of 0.5m in a low flow regime there were significantly fewer lobsters on disease treatment sites (df = 146; t value = -3.95; Adj P = 0.0066).

Repeated-measures ANOVA results indicated that mean distance moved by lobsters differed significantly as a consequence of health status ($P < 0.0001$) and distance from central treatment ($P < 0.0001$), but flow regime was not a significant factor ($P = 0.8331$) (Table 3-6). Within the diseased treatment the movement did not differ significantly between low and high flow rate ($P = 0.6741$) nor did flow rate differ significantly within the non-diseased treatment ($P = 0.2091$). Within the high flow treatment, movement did not differ significantly between the diseased and non-diseased treatment ($P = 0.3389$). Within the low flow treatment, lobsters moved significantly further away from the central treatment shelter on diseased treatment sites ($P = 0.0003$) (Table 3-7) (Figure 3-6).

Within health status, lobster movement was significantly different among the distance from the central treatment (Table 3-8). Within the diseased treatment, movement differed significantly when comparing the original inhabited shelter distance from the central treatment of 0.5m to 1m ($P < 0.0001$) and 0.5m to 2m ($P < 0.0001$). On the other hand, when comparing the original inhabited shelter distance from the central treatment, 1m did not differ significantly from 2m ($P= 0.0506$). Within the original inhabited shelter distance of 0.5m from the central shelter lobsters moved significantly father away from the central treatment at diseased sites ($P < 0.0001$). There were no significant differences in lobster movement from shelters located 1m ($P = 0.8325$) and 2m ($P = 0.9651$) from the central treatment.

Table 3-1. Results determining the detection of PaV1-infected conspecifics. (a) Results from the olfactory treatments only. (b) Results from the visual treatments only

Experiment	n/n	P	1-β
a. Olfaction treatments			
Non-diseased/seawater only	20/10	0.0987	0.432
Diseased/seawater only	05/15	*0.0414	0.617
Diseased/non-diseased	03/18	*0.0015	0.932
Diseased blocked/non-diseased	15/15	1.0000	0.043
b. Visual treatments			
Non-diseased/seawater only	15/15	1.0000	0.043
Diseased/seawater only	14/09	0.4049	0.143
Diseased/non-diseased	14/16	0.8555	0.057

Note: Treatments were tested against random sheltering (50%). P-values denoted with "*" indicate a significant result.

Table 3-2. Results used to determine the range of the PaV1 olfactory avoidance cue. (a) Overall ANOVA results. (b) Multiple comparison test comparing the distance relative to non-diseased and diseased treatments

a. Source	df	Type III SS	F value	P
Treatment	1	1.5	0.00	0.9986
Distance	3	256,985,190.0	186.18	*< 0.0001
Treatment x distance	3	9,667,823.0	6.79	*0.0002
b. Multiple comparison test				
Within shelter	1	6,761,731.0	14.25	*0.0002
0 – 50 cm from shelter	1	661,570.0	1.39	0.2393
50 – 100 cm from shelter	1	107,237.0	0.23	0.6351
100 – 150 cm from shelter	1	2,137,287.0	4.50	*0.0352

Note: P-values denoted with "*" indicate a significant result.

Table 3-3. Results used to determine effect of disease avoidance on the number of lobsters per site over time and under high and low flow regimes

Source	df	F value	P
Time	71	20.17	*< 0.0001
Health status	146	11.42	*0.0009
Flow	146	2.95	0.0879
Distance from stimulus	146	1.19	0.3069
Health status X flow	146	3.63	0.0586
Healthy status X time	146	2.24	0.1097
Flow X time	146	0.84	0.4345
Health status x distance from stimulus	146	0.26	0.7734
Flow X distance from stimulus	146	3.60	*0.0298
Time X health status X distance from stimulus	146	2.28	*0.0248
Healthy status X flow rate X distance from stimulus	146	2.69	0.0715

Note: P-values denoted with "*" indicate a significant result.

Table 3-4. Results used to determine the spatial structuring capabilities of PaV1 relative to the number of lobsters on the site

Comparison within	Comparison between	df	Difference of means	SE	t value	Adj P
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
Comparison						
Disease Low	Non-diseased high	146	-0.34	0.088	-3.88	*0.0009
Disease High	Non-diseased low	146	-0.11	0.101	-1.10	0.6894

Note: This multiple comparison is of health status and flow rate. P-values denoted with "*" indicate a significant result.

Table 3-5. Results used to determine the spatial structuring capabilities of PaV1 relative to the number of lobsters on the site

Comparison within	Comparison between	df	Difference of means	SE	t value	Adj P
Disease	0.5m – 1.0m	146	-0.02	0.064	-0.34	0.9994
	0.5m – 2.0m	146	-0.09	0.064	-1.44	0.7056
	1.0m – 2.0m	146	-0.07	0.064	-1.10	0.8815
Non-diseased	0.5m – 1.0m	146	0.00	0.057	0.04	1.0000
	0.5m – 2.0m	146	-0.03	0.057	-0.54	0.9942
	1.0m – 2.0m	146	-0.03	0.057	-0.59	0.9918
0.5m	Health status	146	-0.25	0.083	-3.05	*0.0314
1m	Health status	146	-0.23	0.083	-2.77	0.0690
2m	Health status	146	-0.19	0.083	-2.32	0.1929

Note: This multiple comparison analysis is of health status and distance from the central shelter. P-values denoted with "*" indicate a significant result.

Table 3-6. Results used to determine the influence of disease avoidance on the juvenile populations structure

Source	df	F value	P
Time	43	2.92	0.0946
Health status	290	17.82	*< 0.0001
Flow	290	0.04	0.8331
Distance from stimulus	290	91.30	*< 0.0001
Health status X flow	290	4.20	*0.0414
Healthy status X time	290	1.44	0.2315
Flow X time	290	0.34	0.5629
Health status X distance from stimulus	290	9.71	*< 0.0001
Flow X distance from stimulus	290	0.54	0.5812
Time X health status X distance from stimulus	290	1.06	0.3777
Healthy status X flow rate X distance from stimulus	290	2.15	0.1186

Note: This analysis was used to determine differences in lobster movement over time. P-values denoted with "*" indicate a significant result.

Table 3-7. Results used to determine the affect of PaV1-infected conspecific avoidance on lobster movement

Comparison within	Comparison between	df	Difference of means	SE	t value	Adj P
Disease	Flow regime	290	-0.25	0.223	-1.13	0.6741
Non-diseased	Flow regime	290	0.31	0.157	1.95	0.2091
Low flow	Health status	290	-0.85	0.206	-4.14	*0.0003
High flow	Health status	290	-0.30	0.178	-1.67	0.3389
Comparison						
Disease low	Non-diseased high	290	-0.55	0.204	-2.68	*0.0383
Disease high	Non-diseased low	290	-0.60	0.181	-3.34	*0.0052

Note: This multiple comparison analysis is of health status and flow rate. P-values denoted with "*" indicate a significant result.

Table 3-8. Results used to determine the affect of PaV1-infected conspecific avoidance on lobster movement

Comparison within	Comparison between	df	Differences of means	SE	t value	Adj P
Disease	0.5m – 1.0m	290	-1.86	0.252	-7.37	*< 0.0001
	0.5m – 2.0m	290	-2.46	0.240	-10.25	*< 0.0001
	1.0m – 2.0m	290	-0.60	0.211	-2.86	0.0506
Non-diseased	0.5m – 1.0m	290	-0.79	0.150	-5.26	*< 0.0001
	0.5m – 2.0m	290	-1.30	0.140	-9.02	*< 0.0001
	1.0m – 2.0m	290	-0.51	0.152	-3.34	*0.0119
0.5m	Health status	290	-1.32	0.231	-5.71	*< 0.0001
1.0m	Health status	290	-0.25	0.208	-1.21	0.8325
2.0m	Health status	290	-0.16	0.191	-0.81	0.9651

Note: This multiple comparison analysis is of health status and distance from the central shelter. P-values denoted with "*" indicate a significant result.

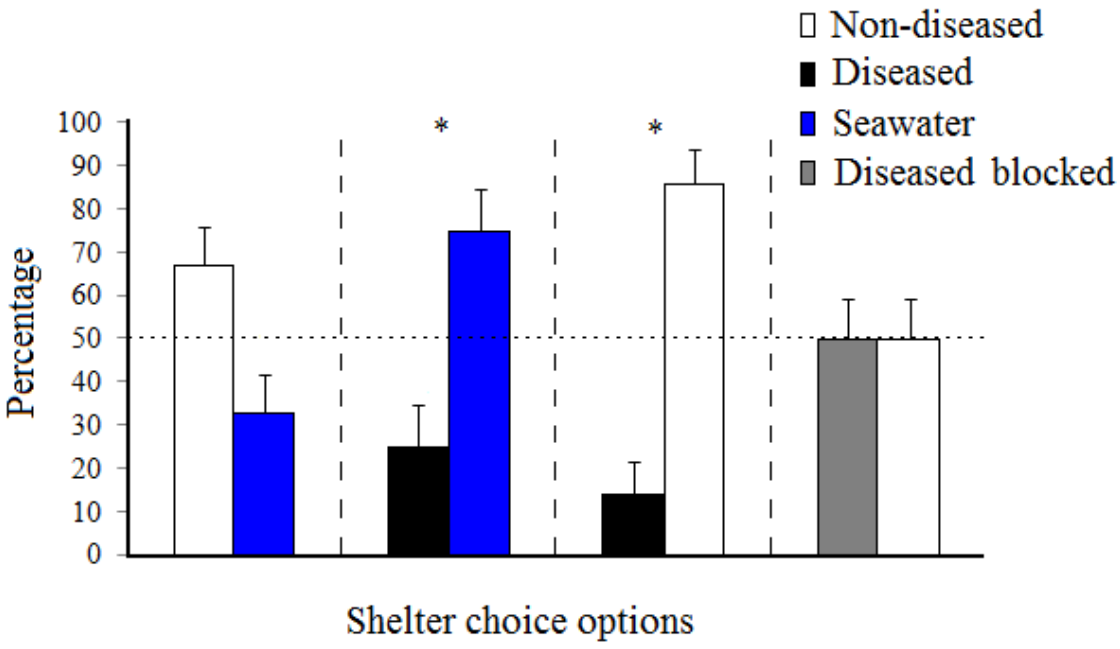

Figure 3-1. The sheltering choice (%) of focal lobsters in Y-maze experiments used to determine the olfactory detection of PaV1-infected conspecifics. The horizontal dotted line represents random sheltering (50%). Within the hash marks, paired bars with an "*"were significantly different from random sheltering. Error bars represent 1 standard error.

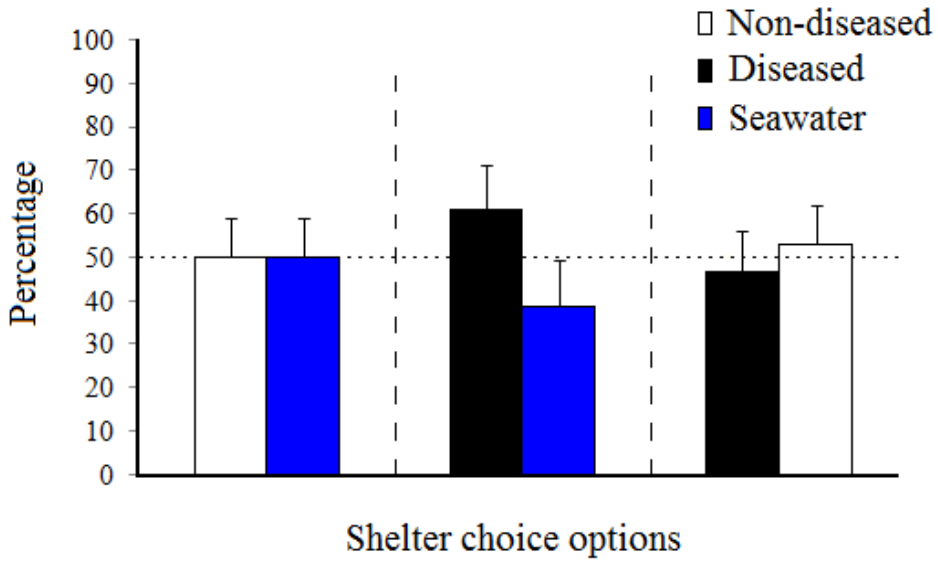

Figure 3-2. The sheltering choice (%) of focal lobsters in Y-maze experiments used to determine the visual detection of PaV1-infected conspecifics. The horizontal dotted line represents random sheltering (50%). Within the hash marks, no treatments were significantly different from random sheltering. Error bars represent 1 standard error.

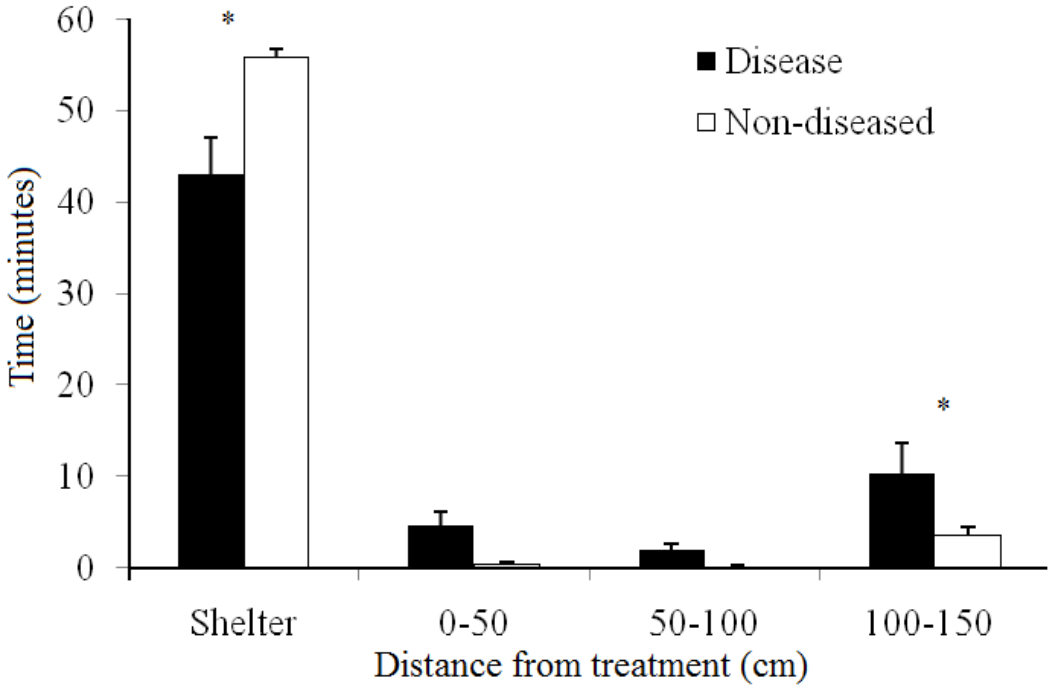

Figure 3-3. The amount of time spent in each location relative to health status in the PaV1-infected conspecific avoidance range experiment. Paired bars with an "*" were significantly different from one another. Error bars represent 1 standard error.

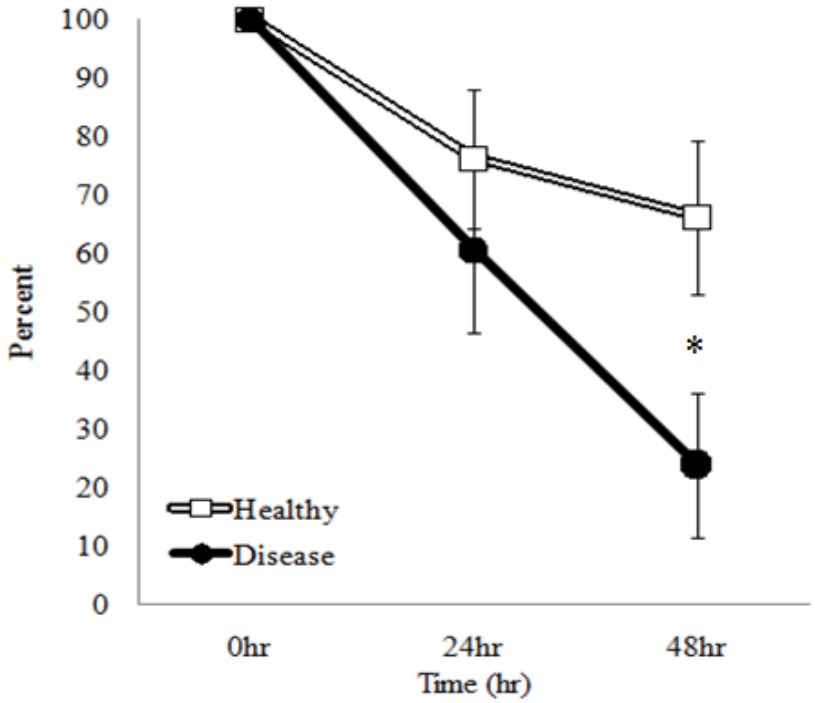

Figure 3-4. The percentage of lobsters remaining on the field sites over time between non-diseased and diseased treatments in the PaV1 population structuring experiments. Standard error bars with an "*" indicate significant differences.

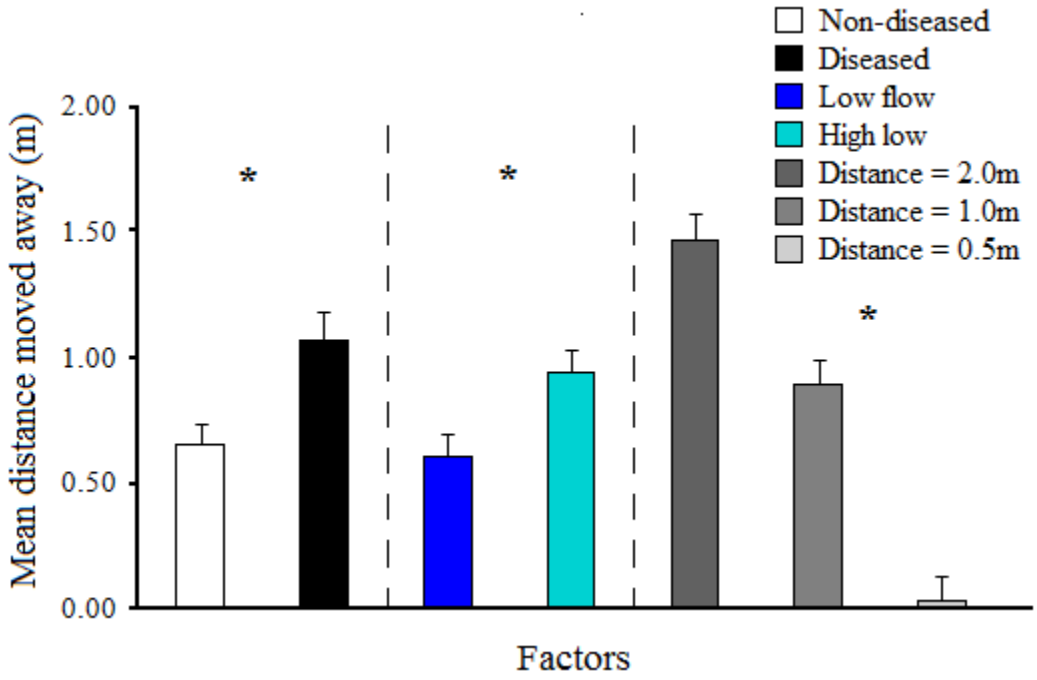

Figure 3-5. The mean distance moved away from central stimulus between the different factors irrespective of the other factors in PaV1 population structuring experiments. Paired bars within the hash marks with an "*" were significantly different from one another. Error bars represent 1 standard error.

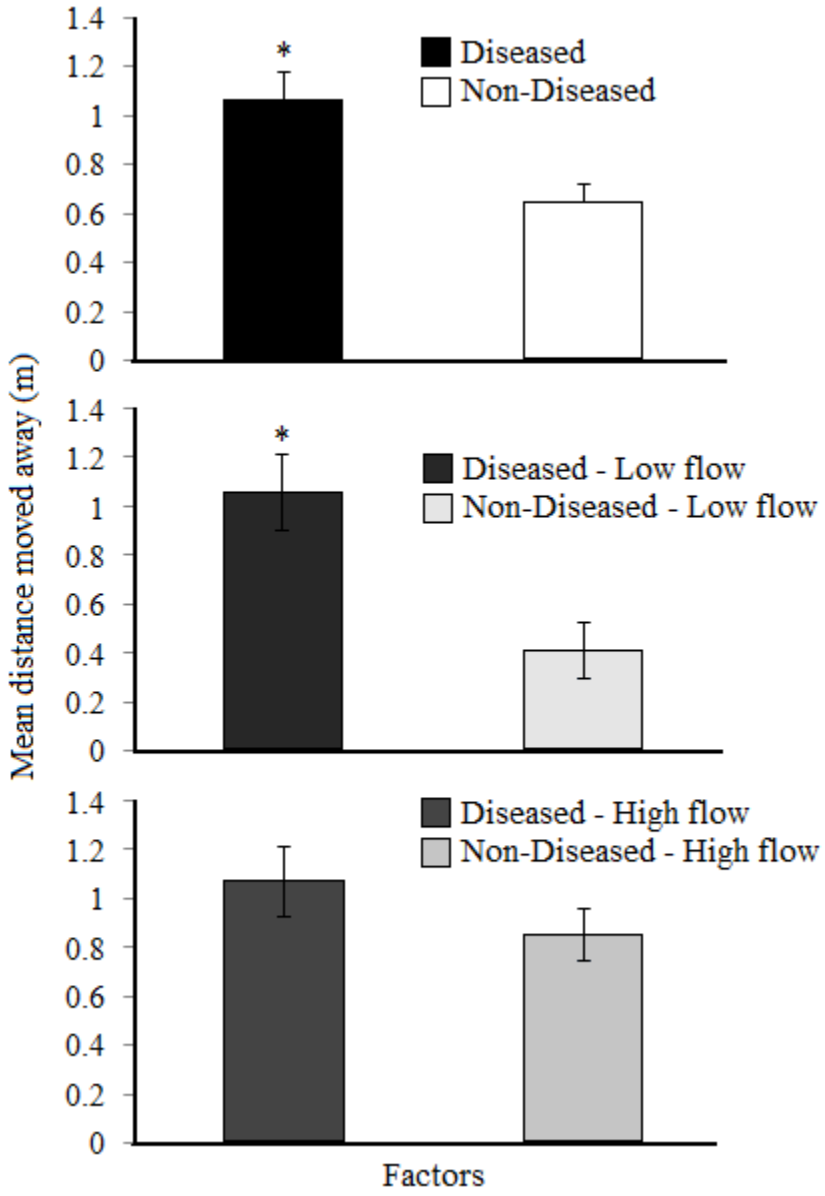

Figure 3-6. The mean distance moved away from central stimulus between the different factors in PaV1 population structuring experiments. A bar with "*" was significantly different than the other treatment. Error bars represent 1 standard error.

CHAPTER 4
DISCUSSION

The avoidance behavior that visibly healthy *P. argus* displays in response to PaV1-infected conspecifics is driven by olfactory cues present in the urine of diseased individuals. Detection of PaV1-infected conspecifics led to spatial structuring of juvenile populations on experimental field sites through, emigration and redistribution. Compounding the affects of disease avoidance on the juvenile population is the influence of differing flow regimes on the detection of PaV1-infected conspecifics, with greater emigration in low flow environments. Effects of emigration and redistribution in shelter-limited habitats could increase juvenile mortality (Behringer and Butler 2010) and subsequently induce a population bottleneck by potentially increasing PaV1 transmission or predation.

Olfaction is a highly efficient mode of habitat assessment in the marine environment used during feeding (Carr and Derby 1986, Finelli et al. 2000), migration/homing events (Herrnkind 1969, Barbin et al. 1998, Mitamura et al. 2005), predator avoidance (Liley 1982, McCormick and Larson 2007), and other species-specific interactions (Ameyaw-Akumfi and Hazlett 1975, Diaz and Theil 2004, Kamio et al. 2008). This efficiency holds true with disease detection as non-diseased lobster cues were more favorable than diseased cues to animals searching for shelter. The source of the avoidance cue was determined to be urine because when the nephropores of infected lobsters were blocked the avoidance behavior ceased. To focal animals searching for suitable shelters, the olfactory cues of diseased animals with blocked nephropores mirrored non-diseased animals. Like the formation of shelter aggregations, many social interactions are also chemically-mediated including individual recognition (Karavanich and Atema 1998 a,b, Zulandt Schneider et al. 2001) and reproduction (Atema and Engstrom 1971, Diaz and Theil 2004, Kamio et al. 2008). The actual cue being detected in the urine is unknown, although it could be

the metabolic waste concentration (Breithaupt and Eger 2002, Atema and Steinbach 2007) or the actual physical PaV1 virus, future work is needed to determine this avoidance driving cue. On the other hand, visual cues do not appear to play a role in PaV1 avoidance. Olfactory detection likely obfuscates the need for visual detection and bimodal signal enhancement was not evident. Olfactory detection of PaV1-infected conspecifics might also dominate because it is sensitive to infected conspecifics even when they are not yet visibly infected or infectious (Behringer et al. 2006).

Although healthy lobsters avoided infected conspecifics, the range of the avoidance response in a laboratory setting could not be determined. During avoidance range experiments, focal lobsters spent significantly more time further away (+1m) from diseased animals than non-diseased animals, but also spent a considerable amount of time sheltered with diseased and non-diseased conspecifics. Compared to PaV1-infected conspecifics detection experiments, the range detection experiment gave focal animals only one shelter choice, of which 86% of focal animals sheltered with the diseased treatment for a period of time. Although animals instinctively avoid diseased individuals, under these experimental conditions it appears that shelter limitation, and presumably the threat of predation, outweighed the threat of disease transmission. Behringer and Butler (2010) found similar sheltering behaviors of *P. argus* within a laboratory setting with focal animals sheltering with diseased animals when only one shelter was provided. While the findings reported herein are possibly due to the inadequate length of the flume, the fact that animals spent significantly more time further away from the diseased treatment than non-diseased treatment, mirrors previous *P. argus* olfactory experiments in which lobsters could exchange social signals over short distances and orient to odor plumes 2 m away (Derby et al. 2001, Shabani et al. 2009). Other factors that could have influence the outcome of this

experiment was the fact that turbulence caused by the flow could have disrupted odor plumes decreasing their readability and the trial time (1h) may have been too short to display altered sheltering behaviors.

Field experiments demonstrated that disease avoidance dramatically altered the spatial structuring of juvenile populations via redistribution and emigration. Not only did diseased treatment sites have fewer lobsters over time, but the remaining animals moved farther away from the diseased lobster. After a diseased conspecific finds its way into the population it would be advantageous for neighboring lobsters to quickly move away. Although the maximum distance was not determined in the laboratory, in the field diseased lobsters had an effect on wild juveniles at least 1m away. These results are consistent with experiments indicating that urine is diluted to 0.1% of its original strength 1m from the source which suggests that specific urine driven behaviors probably only function over short distances (Shabani et al. 2009) compared to non-conspecific driven behavior experiments in which juvenile lobsters were able to chemically detect and avoid octopus predators from up to 2m away (Berger and Butler 2001). Other factors that could have played a role in the animal movement and the reduction of animals at the sites could be the stress caused by handling (induced by divers collecting and tagging animals) and also predation which can be very high on these vulnerable juveniles (Smith and Herrnkind 1992). During surveys no predators were noticed on the sites although they could still be present, but because focal animals at the diseased and non-diseased treatment sites experience the same handling and predation rates, the different in the number of lobsters at the sites can be attributed to the experimental treatments.

Influence of disease on population structuring dynamics was affected by flow, with high flow regimes displaying a lesser effect on movement patterns and emigration. In low flow

environments the presence of a diseased animal significantly altered population structure by inducing emigration, possibly due to the concentrated diseased odor plume diffusing over the habitat without being diluted by turbulence (Finelli 2000). High velocity flow and physical structures downstream of the odor source (concrete shelters) eliminated the affects of disease avoidance, presumably by diluting the odor plume produced by the diseased lobster (Finelli 2000). Reduced olfactory efficiency in high velocity flow could possibly result from turbulence reducing the animal's chance of contacting the diseased odor plume or turbulence disrupting the antennule boundary layer before it can be read (Weissburg and Zimmer-Faust 1993). In areas of daily tidal fluctuations, periods of turbulence could possibly render odor plumes unreadable, but during changes in tidal direction animals could have the opportunity to sense the odor plume and respond.

The small-scale population structuring effects of the avoidance of PaV1-infected conspecifics have similarities to terrestrial and freshwater disease avoidance. For example, black spot disease *Uvulifer* sp., can affect the population structure of the western mosquitofish, *Gambusia affinis* (Tobler and Schlupp 2008). Both healthy and diseased females avoid shoaling with infected conspecifics (Tobler and Schlupp 2008). Similarly, a study on the effects of *Rhopalosiphum padi* virus (RhPV) on the aphid *R. 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). Infected aphids were also found to be more sensitive to a species-specific alarm cue causing them to leave the host plant resulting in increased mortality and a possible decrease in transmission (Ban et al. 2008). Organisms such as *R. padi* and *P. argus*, avoid infected conspecifics through olfactory processes, causing segregation within the population. Segregation is apt to take a heavy toll on

diseased individuals as they have been shown to suffer higher levels of predation, probably due to their lethargic condition (Ban et al. 2008, Behringer and Butler 2010). These examples illustrate that the population structuring affects of disease avoidance is apparent in a broad suite of environments.

Higher levels of predation possibly resulting from disease avoidance could result in a population bottleneck of juveniles in shelter-limited habitats when PaV1 prevalence is high (Herrnkind et al. 1997, Behringer and Butler 2006, Behringer and Butler 2010). In habitats that are shelter-limited naturally or due to habitat degradation (Butler and Herrnkind 1997, Herrnkind et al. 1997), the avoidance of infected animals and the subsequent redistribution and emigration 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 mostly small, vulnerable juveniles (Eggleston et al. 1990, Eggleston et al. 1992, Smith and Herrnkind 1992). The vulnerability of unsheltered juveniles is due to them having a large suite of suitable predators (Eggleston et al. 1990, Smith and Herrnkind 1992, Mintz et al. 1994) and the lack of protection that group defense offers (Zimmer-Faust and Spanier 1987, Eggleston et al. 1990, Smith and Herrnkind 1992). Likewise, non-emigrating animals also could have a risk of increased mortality due to the increased risk of contracting PaV1 from nearby infected conspecifics. Such situations of high disease prevalence and limited shelters could cause juveniles to incur increased mortality due to predation or disease transmission. It would seem maladaptive that disease avoidance would cause an increase in predation on healthy lobsters, but the overall increase in fitness gained by avoiding disease may be greater.

While disease avoidance would seem logical, some organisms are indifferent or even attracted to diseased conspecifics (Han et al. 2008, Bouwman and Hawley 2010). For example, two amphibians, *Bufo boreas* and *Rana cascadae*, were indifferent to conspecifics infected with the pathogen *Batrachochytrium dendrobatidis*, and healthy *R. cascadae* were actually found more often than not aggregated with infected individuals (Han et al. 2008). Bouwman and Hawley (2010) studied the effects of the pathogen *Mycoplasma gallisepticum* on the male house finch, *Carpodacus mexicanus*, and its reaction towards infected conspecifics. Healthy males actually preferred to feed next to overtly infected finches because they were less aggressive and therefore less energetically costly to healthy males; i.e., the energetic cost of feeding near a lethargic infected male was lower (Bouwman and Hawley 2010). Although cases of disease attraction do exist, it is usually induced by an attraction to lethargic conspecifics and therefore less energetically costly to the healthy individual (Bouwman and Hawley 2010).

Although few studies have dealt with the hypothetical implications of parasites or diseases on marine food webs or population structure (Sousa 1991, Thompson et al. 2005, Behringer and Butler 2010), no previous studies have determined the influence of disease avoidance on the spatial structure of a marine population. Pathogens can disrupt nearly every aspect of an animal's life (e.g., reproduction, feeding, social interactions) and through avoidance have the ability to influence population structuring. A number of studies have dealt with disease avoidance and the possible implications on population or community structure (Thompson et al. 2002, Behringer et al. 2006, Ban et al. 2008). Community structure (e.g., host aggregation) can influence the transmission of contact-transmitted pathogens (Shields and Behringer 2004, Wright and Gompper 2005), but host movement due to emigration, disease avoidance, or resource depletion can influence this transmission (Freeland 1979, Wright and Gompper 2005). By

manipulating resources Wright and Gompper (2005) found that the prevalence and species richness of endoparasites within wild raccoons, *Procyon lotor*, increased as the host contact rate increased around clumped resources.

Not only does the ability to detect infectious pathogens influence the community structure, but infected individual behavior can also play a role (Behringer et al. 2006, Ban et al. 2008, Han et al. 2008). Similar to the Caribbean spiny lobster (Behringer et al. 2006), infected individuals of some organisms are still inherently attracted to their conspecifics (Ban et al. 2008, Han et al. 2008). For example the amphibian *B. boreas*, infected with the fungal pathogen, *Batrachochytrium dendrobatidis*, is still attracted to healthy individuals and will actively try to aggregate with them (Han et al. 2008). Likewise, infected individuals of this study species will shelter with infected and healthy animals alike (Behringer et al. 2006). This could possibly result in the aggregation of infected individuals within the population and therefore a decrease in transmission (possible disease sink) but could also result in diseased animals moving into shelters inhabited by non-diseased individuals and transmitting the disease to these individuals. The behavioral response of diseased individuals to diseased olfactory cues needs to be further studied to fully understand how their attraction and movement towards healthy individuals can influence population structuring dynamics.

Conclusion: The ability of visibly healthy lobsters to detect and avoid PaV1-infected conspecifics is driven by chemosensory cues and this can influence the spatial structure of wild juvenile populations through redistribution and emigration. However, local hydrodynamics can alter this effect, presumably by affecting the distribution and detection of odor plumes. Although effective at decreasing disease transmission, disease avoidance could also increase vulnerability to predation, further altering population structure. Clearly disease can play a pivotal role in

animal behaviors and in concert with the physical environment, can be a major structuring force for populations, both demographically and spatially.

LIST OF REFERENCES

Ache, B. W., J. M. Young. 2005. Olfaction: Diverse species, conserved principles. Neuron 48:417-430.

Acquistapace, P., L. Aquiloni, B. A. Hazlett, F. Gherardi. 2002. Multimodal communication in crayfish: Sex recognition during mate search by male *Austropotamobius pallipes.* Canadian Journal of Zoology 80:2041-2045.

Ameyaw-Akumfi, C., and B. A. Hazlett. 1975. Sex recognition in the crayfish *Procambarus Clarkii.* Science 190:1225-1226.

Apfelbach, R, C. D. Blanchard, R. J. Blanchard, R. W. Hayes, and I. S. McGregor. 2005. The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neuroscience & Biobehavorial Reviews 29:1123-1144.

Atema, J. 1986. Review of sexual selection and chemical communication in the lobster, *Homarus americanus*. Canadian Journal of Fisheries and Aquatic Science 43:2283-2390.

Atema, J., and D. G. Engstrom. 1971. Sex pheromone in the lobster, *Homarus americanus.* Nature 232:261-263.

Atema, J., and M. A. Steinbach. 2007. Chemical communication and social behavior of the lobster *Homarus americanus* and other decapods crustacean. Pages 115-144 *in*: J. E. Duffy and M. Thiel editors. Evolutionary ecology of social and sexual systems: Crustaceans as model organisms. Oxford University Press, New York, New York.

Averhoff, W. W., and R. H. Richardson. 1974. Pheromonal control of mating patterns in *Drosophila melanogaster*. Behavior Genetics 4:207-225.

Baldwin, J., and S. Johnsen. 2009. The importance of color in mate choice of the blue crab *Callinectes sapidus*. Journal of Experimental Biology 212:3762-3768.

Bamber, S. D., and E. Naylor. 1997. Sites of release of putative sex pheromone and sexual behavior in female *Carinus maenas* (Crustacea: Decapoda). Estuarine, Coastal and Shelf Science 44:195-202.

Ban, L., E. Ahmed, V. Ninkovic, G. Delp, and R. Glinwood. 2008. Infection with an insect virus affects olfactory behaviour and interactions with host plant and natural enemies in an aphid. Entomologia Experimentalis et Applicata 127:108-117.

Barbin, G. P., S. J. Parker, and J. D. MeCleave. 1998. Olfactory clues play a critical role in estuarine migration of silver-phase American eels. Environmental Biology of Fishes 53:283-291.

Behringer, D. C., and M. J. Butler IV. 2006. Density-dependent population dynamics in juvenile *Panulirus argus* (Latreille): The impact of artificial density enhancement. Journal of Experimental Marine Biology and Ecology 334:84-95.

Behringer, D. C., and M. J. Butler IV. 2010. Disease avoidance influences shelter use and predation in Caribbean spiny lobster. Behavioral Ecology and Sociobiology 64:747-755.

Behringer, D. C., M. J. Butler IV, and J. D. Shields. 2006. Avoidance of disease by social lobsters. Nature 441:421.

Behringer, D. C., M. J. Butler IV, and J. D. Shields. 2008. Ecological and physiological effects of PaV1 infection on the Caribbean spiny lobster (*Panulirus argus* Latreille). Journal of Experimental Marine Biology and Ecology 359:26-33.

Berger, D. K., and M. J. Butler IV. 2001. Octopuses influence den selection by juvenile Caribbean spiny lobster. Marine and Freshwater Research 52:1049-1053.

Bonadonna, F., and G. A. Nevitt. 2004. Partner-specific odor recognition in an Antarctic seabird. Science 306:835.

Bouwman, K. M., and D. M. Hawley. 2010. Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. Biology Letters 6:462-465.

Breithaupt. T., and P. Eger. 2002. Urine makes the difference: Chemical communication in fighting crayfish made visible. The Journal of Experimental Biology 205:1221-1231.

Bushmann, P. J. 1999. Concurrent signals and behavioral plasticity in blue crab (*Callinectes sapidus* Rathbun) courtship. Biological Bulletin 197:63-71.

Bushmann, P. J., and J. Atema. 1997. Shelter sharing and chemical courtship signals in the lobster *Homarus americanus*. Canadian Journal of Fisheries and Aquatic Science 54:647-654.

Butler IV, M. J., D. C. Behringer, and J. D. Shields. 2008. Transmission of *Panulirus argus* virus 1 (PaV1) and its effects on the survival of juvenile Caribbean spiny lobsters. Disease of Aquatic Organisms 79:173-182.

Butler IV, M. J., and W. F. Herrnkind. 1997. A test of recruitment limitation and the potential for artificial enhancement of spiny lobster (*Panulirus argus*) populations in Florida. Canadian Journal of Fisheries and Aquatic Science 54:452-463.

Caldwell, R. L. 1992. Recognition, signaling and reduced aggression between former mates in a stomatopod. Animal Behaviour 44:11-19.

Carr, W., and C. D. Derby. 1986. Behavioral chemoattractants for the shrimp, *Palaemonetes pugio* – identification of active components in food extracts and evidence of synergistic mixture interactions. Chemical Senses 11:49-64.

Carter, C. S., L. L. Getz, L. Gavish, J. L. McDermott, and P. Arnold. 1980. Male-related pheormones and activation of female reproduction in the prairie vole (*Microtus ochrogaster*). Biology of Reproduction 23:1038-1045.

Cate, H. S., and C. D. Derby. 2002. Hooded sensilla homologues: Structural variations of a widely distributed bimodal chemomechanosensillum. Journal of Comparative Neurology. 444:345-357.

Childress, M. J., and W. F. Herrnkind. 1997. Den sharing by juvenile Caribbean spiny lobsters (*Panulirus argus*) in nursery habitat: Cooperation or coincidence? Marine and Freshwater Research 48:751-758.

Childress, M. J., and W. F. Herrnkind. 2001. The guide effect influence on the gregariousness of juvenile Caribbean spiny lobsters. Animal Behaviour 62:465-472.

Cronin, T. W. 1986. Photoreception in marine invertebrates. American Zoologist 26:403-415.

Daniel, P. C., M. Fox, and S. Mehta. 2008. Identification of chemosensory sensilla mediating antennular flicking behavior in *Panulirus argus*, the Caribbean spiny lobster. Biological Bulletin 215:24-33.

Derby, C.D., P. Steullet, A. J. Horner, and H. S. Cate. 2001. The sensory basis of feeding behaviour in the Caribbean spiny lobster, *Panulirus argus*. Marine and Freshwater Resources 52:1339-1350.

Diaz, E. R., and M. Thiel. 2004. Chemical and visual communication during mate searching in rock shrimp. Biological Bulletin 206:134-143.

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

Eggleston, D. B., R. N. Lipcius, D. L. Miller, and L. Coba-Cetina. 1990. Shelter scaling regulates survival of juvenile Caribbean spiny lobster *Panulirus argus.* Marine Ecology Progress Series 62:79-88.

Finelli, C. M. 2000. Velocity and concentration distributions in turbulent odor plumes in the presence of vegetation mimics: A flume study. Marine Ecology Progress Series 207:297-309.

Finelli, C. M., N. D. Pentcheff, R. K. Zimmer, and D. S. Wethey. 2000. Physical Constraints on ecological processes: A field test of odor-mediated foraging. Ecology 83:784-797.

Freeland, W. J. 1979. Primate social-groups as biological islands. Ecology 60:719-728.

George, R. W., and A. R. Main. 1966. The evolution of spiny lobsters (Palinuridae): A study of evolution in the marine environment. Evolution 21:803-820.

Gleeson, R. A., W. Carr, and H. G. Trapido-Rosenthall. 1993. Morphological-characteristics facilitating stimulus access and removal in the olfactory organ of the spiny lobster, *Panulirus argus* – insight form the design. Chemical Senses 18:67-75.

Goldman, J. A., and S. N. Patek. 2002. Two sniffing strategies in Palinurid lobsters. The Journal of Experimental Biology 205:3891-3902.

Han, B. A., P. W. Bradley, and A. R. Blaustein. 2008. Ancient behaviors of larval amphibians in response to an emerging fungal pathogen, *Batrachochytrium dendrobatidis*. Behavioral Ecology and Sociobiology 63:241-250.

Hasler, A. D., and W. J. Wisby. 1951. Discrimination of stream odors by fishes and its relation to parent stream behavior. The American Naturalist 135:223-238.

Hemsworth, R., W. Villareal, B. W. Patullo, and D. L. Macmillan. 2007. Crustacean social behavioral changes in response to isolation. Biological Bulletin 213:187-195.

Herberholz, J. 2007. The neural basis of communication in crustaceans. Pages 72-89 *in*: J. E. Duffy and M. Thiel editors. Evolutionary ecology of social and sexual systems: Crustaceans as model organisms. Oxford University Press, New York, New York.

Herrnkind, W, 1969. Queuing behavior of spiny lobsters. Science 164:1425-1427.

Herrnkind, W. F., M. J. Butler IV, J. H. Hunt, and M. Childress. 1997. Role of physical refugia: Implications from a mass sponge die-off in a lobster nursery in Florida. Marine and Freshwater Research 48:759-769.

Horner, A. J., S. P. Nickles, M. J. Weissburg, and C. D. Derby. 2006. Source and specificity of chemical cues mediating shelter preference of Caribbean spiny lobsters (*Panulirus argus*). The Biological Bulletin 211:128-140.

Horner, A. J., M. J. Weissburg, and C. D. Derby. 2008. The olfactory pathway mediates sheltering behavior of Caribbean spiny lobsters, *Panulirus argus*, to conspecific urine signals. Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology 194:243-253.

Hughes, M. 1996. The function of concurrent signals: Visual and chemical communication in snapping shrimp. Animal Behaviour 52:247-257.

Hurst, J. L. 1990. Urine marking in population of wild house mice *Mus domesticus* Rutty. I. Communication between males. Animal Behaviour 40:209-222.

Kamio, M., M. A. Reidenbach, and C. D. Derby. 2008. To paddle or not: Context dependent courtship display by male blue crabs, *Callinectes sapidus*. The Journal of Experimental Biology 211:1243-1248.

Karavanich, C., and J. Atema. 1998a. Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus*. Behaviour 135:719-730.

Karavanich, C., and J. Atema. 1998b. Individual recognition and memory in lobster dominance. Animal Behaviour 56:1553-1560.

Kleerekoper, H. 1967. Some aspects of olfaction in fishes, with special reference to orientation. American Zoologist 7:385-395.

Larson, J. K., and M. I. McCormick. 2005. The role of chemical alarm signals in facilitating learned recognition of novel chemical cues in coral reef fish. Animal Behaviour 69:51-57.

Laverack, M. S. 1988. The diversity of chemoreceptors. Pages 287-312 *in*: J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga, editors. Sensory biology of aquatic animals. Springer-Verlag, New York, New York.

Liley, N. R. 1982. Chemical communication of fish. Canadian Journal of Fisheries and Aquatic Science 39:22-35.

McClave, J. T., and T. Sincich. 2009. Statistics: Eleventh edition. D. Lynch editor. Pearson Prentice Hall Press, Upper Saddle River, New Jersey.

McCormick, M. I., and J. K. Larson. 2007. Field verification of the use of chemical alarm cues in a coral reef fish. Coral Reefs 26:571-576.

Mintz, J. D., R. N. Lipcius, D. B. Eggleston, and M. S. Seebo. 1994. Survival of juvenile Caribbean spiny lobster: Effects of shelter size, geographic location and conspecifics abundance. Marine Ecology Progress Series 112:255-266.

Mitamura, H., N. Arai, W. Sakamoto, Y. Mitsunaga, H. Tanaka, Y. Mukai, K. Nakamura, M. Sasaki, and Y. Yoneda. 2005. Role of olfaction and vision in homing behavior of black rockfish *Sebastes inermis*. Journal of Experimental Marine Biology and Ecology 322:123-134.

Moore, A. J. 1997. The evolution of social signals: Morphological, functional, and genetic integration of the sex pheromone in *Nauphoeta cinerea.* Evolution 51:1920-1928.

Nevitt, G., N. D. Pentcheff, K. J. Lohmann, and R. K. Zimmer. 2000. Den selection by the spiny lobster *Panulirus argus*: Testing attraction to conspecific odors in the field. Marine Ecology Progress Series 203:225-231.

O'Brien, D. P. and D. A. Ritz. 1988. Escape responses of gregarious mysids (Crustacea: Mysidacea): Towards a general classification of escape responses in aggregated crustaceans. Journal of Experimental Marine Biology and Ecology 116:257-272.

Raethke, N., A. B. MacDiarmid, and J. C. Montgomery. 2004. The role of olfaction during mating in the southern temperate spiny lobster *Jasus edwardsii*. Hormones and Behavior 46:311-318.

Ratchford, S. G., and D. B. Eggleston. 1998. Size- and scale-dependent chemical attraction contribute to an ontogenetic shift in sociality. Animal Behaviour 56:1027-1034.

Rebach, S. 1996. Role of prey in food recognition by rock crabs, *Cancer irroratus* Say. Journal of Chemical Ecology 22:2197-2207.

Ritz, D. A. 2000. Is social aggregation in aquatic crustaceans a strategy to conserve energy? Canadian Journal of Fisheries and Aquatic Sciences 57:59-67.

Rosell, F., and J. I. Sanda. 2006. Potential risks of olfactory signaling: The effect of predators on scent marking by beavers. Behavioral Ecology 17:897-904.

Schratwieser, J. 1999. The impact of resident and transient predators on the population dynamics of juvenile Caribbean spiny lobster (*Panulirus argus*) in Florida Bay, Florida. Thesis. Old Dominion University, Norfolk, Virginia.

Schmitt, B. C., and B. W. Ache. 1979. Olfaction: Responses of a decapods crustacean are enhanced by flicking. Science 205:204-206.

Seehausen, O., and J. J. M. van Alphen. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). Behavioral Ecology and Sociobiology 42:1-8.

Shabani, S., M. Kamio, and C. D. Derby. 2009. Spiny lobsters use urine-borne olfactory signaling and physical aggressive behaviors to influence social status of conspecifics. The Journal of Experimental Biology 212:2464-2474.

Shields, J. D., and D. C. Behringer. 2004. A new pathogenic virus in the Caribbean spiny lobster *Panulirus argus* from the Florida Keys. Diseases of Aquatic Organisms 59:108-118.

Smith, K. N., and W. F. Herrnkind. 1992. Predation on early juvenile spiny lobsters *Panulirus argus* (Latreille): Influence of size and shelter. Journal of Experimental Marine Biology and Ecology 157:3-18.

Smith, W. J. 1969. Messages of vertebrate communication. Science 165:145-150.

Sousa, W. P. 1991. Can models of soft-sediment community structure be complete without parasites? American Zoologist 31:821-830.

Stacey, N. 2003. Hormones, pheromones and reproductive behavior. Fish Physiology and Biochemistry 28:229-235.

Thompson, K. G., R. B. Nehring, D. C. Bowden, and T. Wygant. 2002. Response of rainbow trout *Oncorhynchus mykiss* to exposure to *Myxobolus cerebralis* above and below a point source of infectivity in the upper Colorado River. Disease of Aquatic Organisms 49:171-178.

Thompson, R. M., K. N. Mouritsen, and R. Poulin. 2005. Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. Journal of Animal Ecology 74:77-85.

Tobler, M., and I. Schlupp. 2008. Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei). Environmental Biology of Fishes 81:29-34.

Weissburg, M. J., and R. K. Zimmer-Faust. 1993. Life and death in moving fluids: Hydrodynamic effect on chemosensory-mediated predation. Ecology 74:1428-1443.

Wilson, E. O. 1965. Chemical communication in the social insects. Science 149:1064-1071.

Wilson, E. O. 1975. Sociobiology: The new synthesis. Harvard University Press, Cambridge, Massachusetts.

Wright, A. N., and M. E. Gompper. 2005. Altered parasite assemblages in raccoons in response to manipulated resource availability. Oecologia 144:148-156.

Zimmer-Faust, R. K. 1993. ATP: A potent prey attractant evoking carnivory. Limnology and Oceanography 38:1271-1275.

Zimmer-Faust, R. K., and E. Spanier. 1987. Gregariousness and sociality in spiny lobsters: Implications for den habitation. Journal of Experimental Marine Biology and Ecology 105:57-71.

Zimmer-Faust, R. K., J. E. Tyre, and J. F. Case. 1985. Chemical attraction causing aggregation in the spiny lobster, *Panulirus interruptus* (Randall), and its probable ecological significance. Biological Bulletin 169:106-118.

Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: The effects of urine release on fight dynamics. Behaviour 138:137-153.

BIOGRAPHICAL SKETCH

Josh grew up in a no-stoplight town on the foothills of the Missouri Ozarks. He graduated from Russellville High School with an avid interest in the outdoors which he cultivated through his early years growing up and working on the family farm. He furthered his education by attending the University of Missouri - Columbia during which he was employed by the Missouri Department of Conservation. At the Conservation Department, he worked with the endangered Niangua darter (*Etheostoma nianguae*) to determine the health of the population and also worked on understanding the impacts of stream erosion and human induced stream degradation on pristine Ozark streams. Josh graduated in May 2009 with a bachelor's of Fisheries and Wildlife and is still a proud alumnus of the University of Missouri. His loathing of the bitter Missouri winters and his interest in the ecology of marine systems helped him find his way to the sunny University of Florida where he completed his master's degree with Dr. Donald Behringer in the Summer of 2011.