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The Ecological Ramifications of Disease and Density in the Caribbean Spiny Lobster, *Panulirus argus*

Donald C. Behringer Jr.
Old Dominion University

THE ECOLOGICAL RAMIFICATIONS OF DISEASE AND DENSITY IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*

By

Donald C. Behringer, Jr.
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Approved by:

Mark J. Butler, IV (Director)

Kent E. Carpenter (Member)

Jeffrey D. Shields (Member)

ABSTRACT

THE ECOLOGICAL RAMIFICATIONS OF DISEASE AND DENSITY IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*

Donald C. Behringer, Jr.
Old Dominion University, 2002
Director: Dr. Mark J. Butler, IV

In 1999, I discovered the first virus known to be pathogenic to any species of lobster. HLV-PA is a pathogenic herpes-like virus that infects juvenile Caribbean spiny lobster, *Panulirus argus*, in the waters off south Florida (USA), and it alters the behavior and ecology of this species in fundamental ways. Gross signs of HLV-PA infection are lethargy, morbidity, cessation of molting, and discolored, "milky" hemolymph that does not clot. HLV-PA infects the hemocytes of host lobsters, specifically the hyalinocytes and semi-granulocytes, but not the granulocytes. When hemolymph from infected donors was injected into healthy juvenile lobsters, 90% of the healthy individuals became infected within 80 days. In another set of laboratory trials, 40% of the juvenile lobsters that ingested conspecific tissue infected with HLV-PA developed the disease, and in a third experiment wherein transmission by contact or waterborne means was tested, 63% of the lobsters <30 mm carapace length (CL), 33% of lobsters 30-40 mm CL and 10% of lobsters 40-50 mm CL became infected within 80 days.

In field surveys from 2000-2001, up to 40% of the juveniles at each of twelve sites (mean = 8%) had the disease. The disease was most prevalent (mean = 16%) among the smallest juveniles (i.e., < 20 mm CL) and, thus far, appears limited to juveniles. However,

all of the surveys of disease prevalence are based on gross, visual signs of late stages of infection, and are, therefore, conservative estimates. A diagnostic tool to assess infection at earlier stages has not yet been developed.

Field observations and laboratory experiments indicate that healthy juvenile lobsters avoid diseased conspecifics, which is only the second report of such behavior in any animal. The prevalence of the disease in wild lobster populations is not correlated with population density, even when lobsters were experimentally concentrated at sites with artificial shelters. Moreover, enhanced density does not appear to have a detrimental effect on population dynamics such as nutritional condition and short-term residency, likely due to their normal gregariousness. Thus, juvenile spiny lobsters appear to have developed remarkable contradictory behaviors, avoidance of infected conspecifics and gregariousness, both of which may ultimately enhance survival of uninfected lobsters.

This dissertation is dedicated to the loves of my life:

My wife, Dianne, whose love and support I cherish,
and
My daughter, Olivia, whose smile makes it all worthwhile

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I owe a debt of gratitude to numerous people for their inspiration, guidance, brute force labor and not least of all, comradery. The road has been a long one, with numerous hairpin turns and dead-ends, but a trail worth following. Along the way I have experienced and learned a great deal and developed numerous relationships to last a lifetime. To all I will mention and may fail to include, I appreciate every last one of you; you have helped make me who I am.

I first thank my parents, for they not only nurtured me through my youth but also instilled in me the values and ideals from which I base my life. Dianne Behringer, my wife and former fellow graduate student, was essential to this dissertation coming to fruition. Her support and encouragement helped me in times of despair and in times of jubilation. She is my foundation.

Credit for my basic love of science is undoubtedly owed to my father, Donald Sr., who is a scientist in its purest definition, but Louis Guilette, at the University of Florida, truly inspired me to pursue graduate school with a passion. He is enthralled by what he does and it infuses all those around him. My advisor, Mark Butler, has inspired me in much the same way. He lives and breathes science. He has been a model mentor, both scientifically and personally. I am grateful to have had him as an advisor, a colleague and not the least of all, a friend (oh, and thanks for the financial support, too).

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project. He gave not only his time and advice, but financial support as well. I also thank my committee members, past and present for their advice and guidance: Kent Carpenter, Daniel Dauer, Cynthia Jones, Anthony Provenzano, Simon Therrold, and John Holsinger.

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Lastly, I thank William Herrnkind, from whose scientific loins most of those listed here ultimately sprang. His love for the sea, whether studying it, fishing it, or eating it, is contagious.

May the rum, coke and lime continue to inspire the science to pour out of us!

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CHAPTER I

INTRODUCTION

There is a growing interest and implementation of habitat enhancement programs designed to boost field populations and catch of lobsters throughout the world (Fee 1986, Cruz and Philips 2000, Briones et al. 2000). Most artificial structures employed in such programs have been designed by fishermen and serve to concentrate lobsters for ease of capture. However, appropriately designed and placed structures may also be used to alleviate demographic bottlenecks in juvenile populations (Werner and Gilliam 1984, Fogarty and Idoine 1986, Wahle and Steneck 1991, Childress and Herrnkind 1994, Lozano-Alvarez et al. 1994, Beck 1995, 1997, Herrnkind et al. 1997*a*, Butler and Herrnkind 1997). The impact that artificially enhanced population density has on lobster population dynamics such as local (i.e., short-term) residency or nutritional condition is unknown.

Experimental field studies of potential demographic bottlenecks in the recruitment of Caribbean spiny lobster (*Panulirus argus*) have successfully used concrete partition blocks designed to mimic natural crevice shelters to enhance local densities of juvenile lobsters (Butler and Herrnkind 1997, Herrnkind et al. 1997*a*). Using these structures in arrays of low and high abundance, I examined the impact of lobster density on the short-term residency and nutritional condition of individuals dwelling on hard-bottom sites in

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The journal model for this dissertation was *Ecology*.

the Florida Keys, FL (USA). During the course of that study, I discovered a previously unknown disease that afflicts juvenile spiny lobster (Shields and Behringer, *in press*).

Although the effects of density on the incidence of disease in cultured or confined aquatic organisms is well documented (e.g., Getchell 1995, LaPatra 1996, Kautsky et al. 2000, Wu et al. 2001), the impact in the field of artificially enhanced density on disease prevalence is limited to sedentary organisms such as bivalve molluscs (e.g., Kraeuter et al. 1998, Ford et al. 2002). Thus, I was afforded the opportunity to investigate both a previously unknown disease infecting lobsters and the impact of enhanced density on disease prevalence in the field.

HLV-PA, the designation given to this herpes-like virus, is a pathogenic blood-borne virus. Gross signs of infection were lethargy, morbidity, cessation of molting, and discolored "milky" hemolymph (i.e., blood) that does not clot. Furthermore, the infection caused abnormal behavior in lobsters. Juvenile Caribbean spiny lobsters are normally gregarious, co-occupying shelters during the day and foraging at night (Berrill 1975, Eggleston and Lipcius 1992, Childress and Herrnkind 1994, 1996, Ratchford and Eggleston 1998, 2000). To the contrary, infected lobsters in the field were almost invariably solitary.

Many studies have described behavioral alterations in a host infected with a pathogen or parasite (see Hart 1988, 1990, Moore and Gotelli 1990, Poulin 1995 for review). Others have investigated the impact of pathogens and parasites on mating systems and sexual selection (Hamilton and Zuk 1982, Kennedy et al. 1987, Kavaliers and Colwell 1995, Lopez 1998, Penn and Potts 1998). A study by Kiesecker et al. (1999) is the only one to demonstrate a behavior by healthy individuals, avoidance of infected

conspecifics that could potentially reduce infection risk. Similarly, the HLV-PA disease affects the social behavior of the healthy lobster, a change that may be adaptive in reducing the risk of infection in healthy individuals.

In summary, this study describes the pathology of a previously unknown disease, while simultaneously addressing the impact of artificially enhanced density on population parameters including disease prevalence. My objectives were to: (i) describe the etiological agent and its pathology, (ii) determine potential mode(s) of disease transmission, (iii) document altered behaviors in healthy lobsters that may reduce their risk of infection, and (iv) investigate the impact of artificially enhanced lobster density on nutritional condition, short-term residency and disease prevalence.

CHAPTER II

A PATHOGENIC HERPES-LIKE VIRUS IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*, FROM FLORIDA

Introduction

The Caribbean spiny lobster supports extensive commercial and recreational fisheries from Florida, throughout the Caribbean and into northern Brazil. In Florida, it is the most valuable fishery with approximately 90% of the harvest coming from the Florida Keys (FMRI 2001). Furthermore, an estimated 90% of the adult stock in the Florida Keys is landed each year (Hunt 2000). Concerns regarding the sustainability of this heavily fished resource have often targeted overexploitation or degradation of habitat. Few diseases have been identified at a level to raise concern, and none of these appear to have had a negative impact on the population.

Palinurid lobsters in the genera *Panulirus* spp., *Palinurus* spp. and *Jasus* spp. have few reported diseases (for review, see Evans and Brock 1994, Evans et al. 2000). Conclusive viral infections have never been previously demonstrated. Shell disease from chitinoclastic bacteria can cause lesions around the tail and uropods of infected animals resulting in poor marketability (Alderman 1973; Iversen and Beardsley 1976; Sinderman and Rosenfield 1976; Booth 1988). Systemic infections of *Vibrio* spp. have occasionally developed in lobsters subjected to increased temperature, holding stress, or poor water quality (Chong and Chao 1986; Diggles et al. 2000). A presumed bacterial infection called hepatopancreatic disease occurred in larval lobsters used in life history studies, and

the condition was treated with streptomycin (Kittaka and Abrunhosa 1997). Filamentous bacteria, presumably *Leucothrix mucor,* indicative of poor water quality or stress, have been observed on the gills and eggs of *Jasus edwardsii* (Shields, unpubl. data). Additionally, in experimental infections, *Aerococcus viridans*, the causative agent of gaffkemia in clawed lobsters, is pathogenic to *Panulirus interruptus* (Schapiro et al. 1974) and may occur naturally in *Panulirus argus* (Bobes et al. 1988). The later stages of the infection caused "red tail" in clawed lobsters, a syndrome quite different from that observed in viral infections. Fungal infections have been reported on the carapace (Alderman 1973; McAleer cited in Evans et al. 2000), gills (cf. *Didymaria* spp., *Penicillium* spp. – Sordi 1958; B. Diggles, NIWA, NZ, pers. com.) and in larvae of palinurids (Kitancharoen and Hatai 1995). A microsporidian was pathogenic in the muscles of *P. argus, Panulirus cygnus* and *Panulirus ornatus,* but infections were extremely rare (Bach and Beardsley 1976; Dennis and Munday 1994). At least three helminths use spiny lobsters as intermediate hosts. A microphallid trematode infects the ovaries of adult *P. cygnus* (Deblock et al. 1990); a tetraphyllidean cestode occurs in the foregut of several species of spiny lobsters from the Great Barrier Reef (Shields unpubl. data); and a nematode infects the larvae and juveniles of *J. edwardsii* (Brett cited in Booth 1988). Finally, at least two predatory nemerteans, *Carcinonemertes* spp. (Campbell et al. 1990; Shields and Kuris 1990) and amphipods, cf. *Parapleustes* spp. (Shields, personal observation), infest the egg clutches of at least three species of spiny lobsters.

Here I report the first naturally occurring pathogenic virus to be identified from a lobster, specifically the Caribbean spiny lobster (*P. argus*). In 1999 and 2000, while

sampling juvenile spiny lobster populations in the Florida Keys, I discovered lethargic, moribund animals whose hemolymph failed to clot and appeared "thin" and "milky" rather than its normally transparent color. The hemolymph was negative for Gram-negative bacteria, but the histopathology showed nuclear hypertrophy with diffuse Cowdry-type A viral inclusions in infected hemocytes. In heavily infected individuals, virtually all of the hyalinocytes and semigranulocytes of the host were destroyed. My objective in this chapter was to identify the causative agent and describe the histopathology of the infection within juvenile spiny lobster.

Methods

I collected juvenile spiny lobsters from 14 sites just north of the middle and lower Florida Keys. Each site was located in hard-bottom habitat, the preferred nursery habitat of juvenile spiny lobsters in the Florida Keys (Butler et al. 1995, Herrnkind et al. 1997*b*). Sites were surveyed by two divers seasonally during the winter (January-March) and summer (June-August). During the surveys, divers used hand-nets to capture all of the lobsters that they encountered within each $625m^2$ site. Healthy animals were returned to their habitat after determination of their sex and size, and after any injuries noted. Moribund animals were taken to the laboratory for observation and confirmation of disease. To verify the presence of the virus, hemolymph and other tissues from several lobsters were fixed and processed for histology as described below.

Initially, I dissected moribund lobsters obtained in the field (n = 4), along with two healthy lobsters. For histological examination of healthy and potentially diseased tissues, I dissected out hepatopancreas, heart, gill, muscle, foregut, hindgut, and, in some

cases, hemopoietic tissues from each lobster and fixed them separately in 10% neutral buffered formalin. The tissues from a few lobsters were fixed with Bouins solution. All of the tissues were then shipped to Dr. Jeffrey D. Shields at the Virginia Institute of Marine Science where they were processed through routine paraffin procedures using Harris hematoxylin and eosin Y (as in, Humason 1979).

Several live lobsters were shipped to Shields for TEM analysis. Tissues were processed by Shields as follows: the hepatopancreas, connective tissue, gill, and hemopoietic tissue from four infected and one uninfected lobster were prepared for transmission electron microscopy (TEM) using 3% glutaraldehyde in 0.2 M sodium cacodylate buffer. Similar tissues from two different infected and one different control animal were fixed in 3% glutaraldehyde containing 0.2 M sodium cacodylate augmented with 30 mg ml^{-1} NaCl and 20 ug ml^{-1} $CaCl_2$, at pH 7.0 as per Factor and Naar (1985). The latter gave superior results for visualizing viral and host cell morphologies. After fixation, tissues were washed three times in buffer and post-fixed in 1% osmium tetroxide in buffer. Samples were then processed through an ethanol dehydration, *en bloc* stained with uranyl acetate, dehydrated further with propylene oxide, infiltrated through several changes of propylene oxide in various ratios with Spurr's resin, and finally embedded in Spurr's resin. Sections were cut on a Reichert-Jung ultramicrotome E, processed through a routine lead citrate stain, and observed with a Zeiss CEM-902 TEM.

For diagnosis of the virus in hemolymph, I stained blood samples with either Harris hematoxylin and eosin, or with Castañeda's methylene blue protocols (Humason 1979). Briefly, hemolymph was drawn into iced (0 °C) 10% neutral-buffered formalin at a ratio of 5:1 or 10:1 fixative to hemolymph. Fixed samples were stored at 4 °C. For

processing, one to two drops of fixed hemolymph was smeared onto a poly-l-lysine-coated microslide which was then air dried, fixed in 100% methanol and stained using minor modifications to the protocols.

Results

Light microscopy

Heavily infected lobsters exhibited lethargy, an inability to right themselves, infrequent tremors, and milky or chalky hemolymph that failed to clot. Cellular debris and exudates were apparent in the hemolymph of infected animals in late stages of the disease but not in animals with early infections. In heavily infected lobsters, virtually all of the hyalinocytes and semigranulocytes were infected or destroyed (Fig. 1). In the hemolymph, only hyalinocytes and semigranulocytes exhibited alterations due to viral infections; granulocytes were not infected (Fig. 2-5). Altered hemocytes were enlarged, possessed densely staining bands of emarginated chromatin and their nuclei exhibited marked hypertrophy (Fig. 2-4). Heavily infected cells frequently exhibited eosinophilic Cowdry Type-A inclusions in their nuclei. Connective tissue cells were more noticeably infected in heavily infected lobsters (Fig. 3). Pycnotic nuclei, possibly indicative of localized ischemia, were common in moderate and heavy infections (Fig. 3), but karyorrhexis was uncommon.

The following cells and tissues were examined for viral infections: hemocytes, hepatopancreatic tubules, epithelia of the hepatopancreas, fixed phagocytes, gills, gill podocytes, heart, pericardium, connective tissues surrounding the hindgut, the hindgut, and hemopoietic tissues. In heavily infected lobsters, the soft connective tissues in the

hepatopancreas exhibited a marked decline in or even absence of reserve inclusion (RI) cells suggesting a loss of glycogen reserves. RI cells, which vary in relation to molt stage, were abundant in most of the uninfected animals, but virtually all of the virally-infected animals lacked significant reserve inclusions. The hepatopancreas did not exhibit direct lysis, but the organ may have experienced shrinkage as the hemal sinuses were frequently enlarged (Fig. 1-2). Indeed, in heavy infections, the fixed phagocytes and blood vessels were obliterated and the surrounding connective tissues either necrotic or obliterated.

Electron microscopy

The virus showed a distinct predilection for host hyalinocytes and semigranulocytes (Fig. 5-6). Virions, loose aggregates of virions, and virogenic stroma were diffusely distributed around the inner periphery of the nuclear membrane of the infected hemocytes. Nuclear hypertrophy was extreme with the entire nuclear envelope of many infected cells extended to the limit of the plasma membrane. Heavily infected cells frequently possessed loose matrices or aggregates of virions in the cytoplasm (Fig. 6, 12). In heavily infected lobsters, virions were free in the hemolymph and occasionally formed loose aggregates within the perforated membranes of the fixed phagocytes in the hemal sinuses of the hepatopancreatic tubules (Fig. 7), a collection point for viral particles in other crustaceans (Johnson 1980).

The viral agent was an icosahedral, herpes-like DNA virus (HLV-PA) with a nucleocapsid of approximately 187 nm $\pm$ 15 nm (sd) and nucleoids approximately 113 nm $\pm$ 12 nm (sd) (Fig. 8). The capsid had an electron-lucent inner layer and an electron-dense outer layer on which there were possible external projections when located extracellularly (Fig. 8). With the sodium cacodylate buffer augmented with sodium

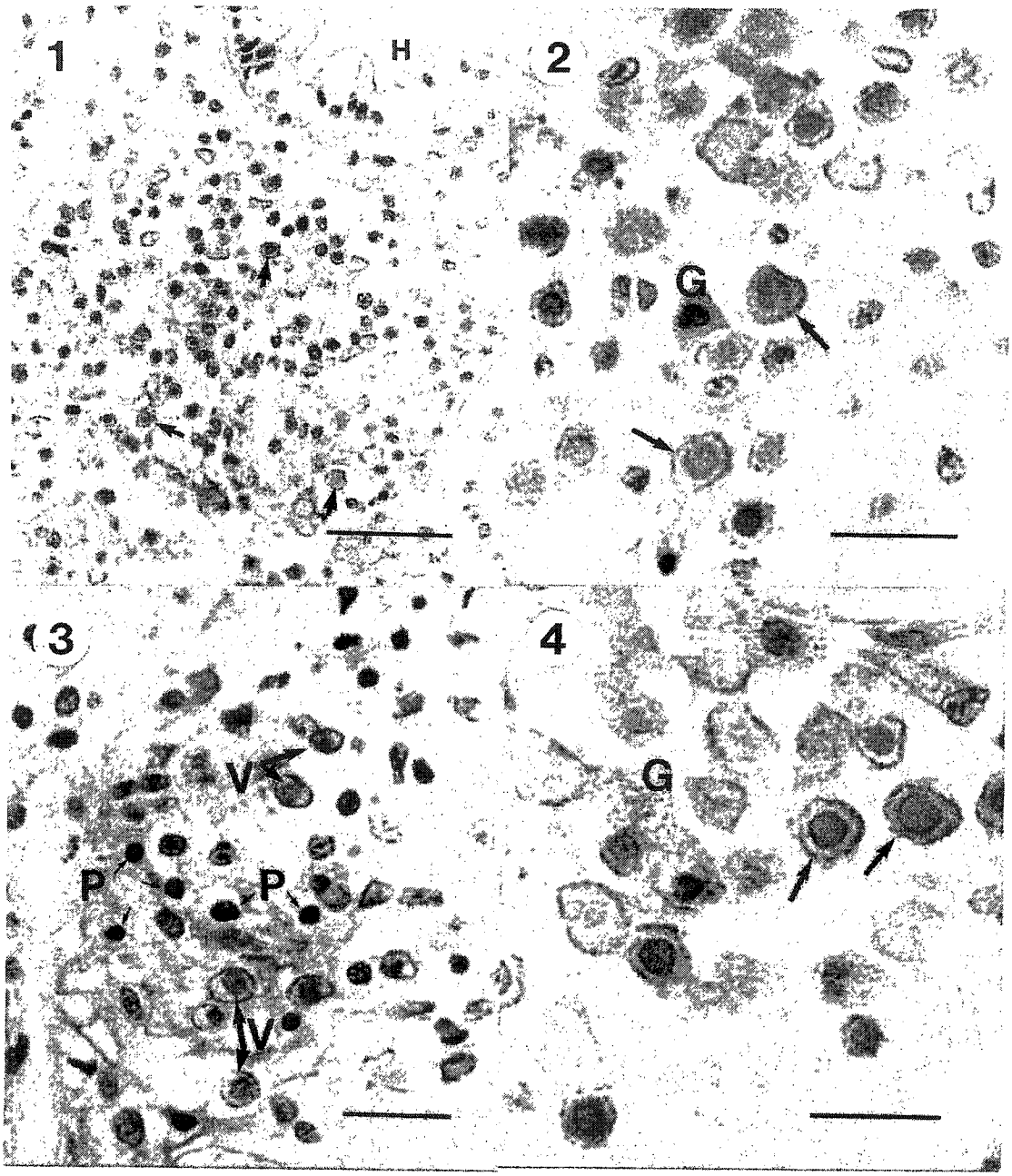

FIG. 1. Hemal sinus adjacent to a hepatopancreatic tubule (H). Infected hemocytes (arrow) are abundant. Connective tissues, reserve inclusion cells and fixed phagocytes have been obliterated. Bar = 150 μm.

FIG. 2. Infected hemocytes (arrow) showing hypertrophied nuclei with emarginated chromatin and diffuse nucleoplasms. Granulocytes (G) are not infected. Bar = 50 μm.

FIG. 3. Infected (V) and pycnotic (P) cells of the soft connective tissues surrounding the hind gut. Bar = 50 μm.

FIG. 4. Infected hemocytes (arrow) showing hypertrophied nuclei with emarginated chromatin and diffuse, fibrillar nucleoplasms. Granulocytes (G) were not infected. Bar = 50 μm.

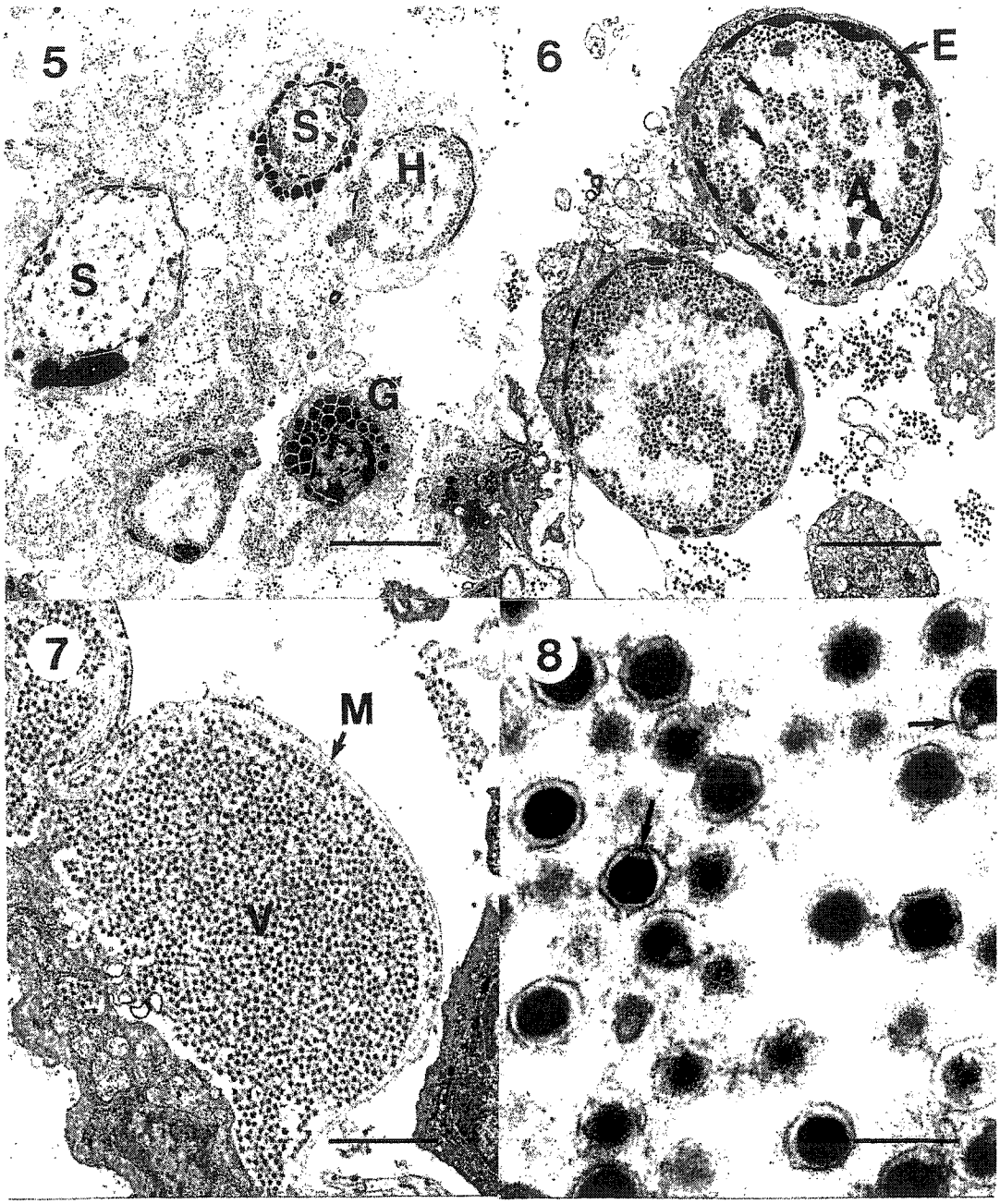

FIG. 5. Infected hyalinocytes (H) and semigranuloctes (S) exhibiting hypertrophied nuclei, emarginated chromatin and juxtanuclear mitochondria. Uninfected granulocyte (G) shown with numerous electron dense granules and normal nucleus. Bar = 10 μm.

FIG. 6. Infected hemocytes showing emarginated chromatin (E), loose aggregates of virions in the nucleoplasm (arrow) and electron dense whorls within the virogenic stroma (A). Bar = 5 μm.

FIG. 7. Matrix of virions (V) aggregated within the perforated membrane of the fixed phagocytes overlying endothelial cells of a hemolymph vessel. Bar = 3 μm.

FIG. 8. Detail of virions from Fig. II.7 showing icosahedral form, cylinder within the toroid (arrow) of the nucleoid surrounded by a bilayered capsid wall. Bar = 250 nm.

chloride and calcium chloride, the nucleoids possessed an internal cylinder surrounded by a toroid structure similar to the classical toroid of the Herpesviridae. The toroid structure was not apparent when tissues were fixed in the glutaraldehyde with the unmodified sodium cacodylate buffer. There was no apparent envelope surrounding the nucleocapsids in the cytoplasm nor was there an envelope surrounding virions outside the cell (e.g., Fig. 8).

Viral assembly of HLV-PA occurred entirely within the nucleus of the host cell (Figs. 9-11). In some cases, apparent viral assembly sites occurred as elongate, electron dense, rod-like elements arising from or adjacent to the emarginated, coalesced chromatin (Fig. 9). Icosahedral nucleocapsids appeared as if budding from the apex of the rod (Fig. 9), and capsid formation occurred along the rod-like elements or within granular matrices prior to the budding or coalescence of the nucleoid (Fig. 10). However, in most cases, presumptive assembly sites appeared as whorls of electron dense material with short, electron dense, rod-like elements with adherent capsid material arising from the whorls (Fig. 11). Uncapsidated nucleoids were also present in heavily infected cells (Fig. 11). Unlike the herpes viruses, there was no envelope formation around the nucleocapsid during migration through the nuclear envelope, nor was there an envelope present during migration through the cytoplasmic membrane (Fig. 8, 12). Virions migrated through the nuclear envelope into the cytoplasm and formed loose aggregates in the cytoplasm prior to cell lysis (Fig. 12). In heavy infections, virions occurred freely within the hemal sinuses of the hepatopancreas (Figs. 6-7).

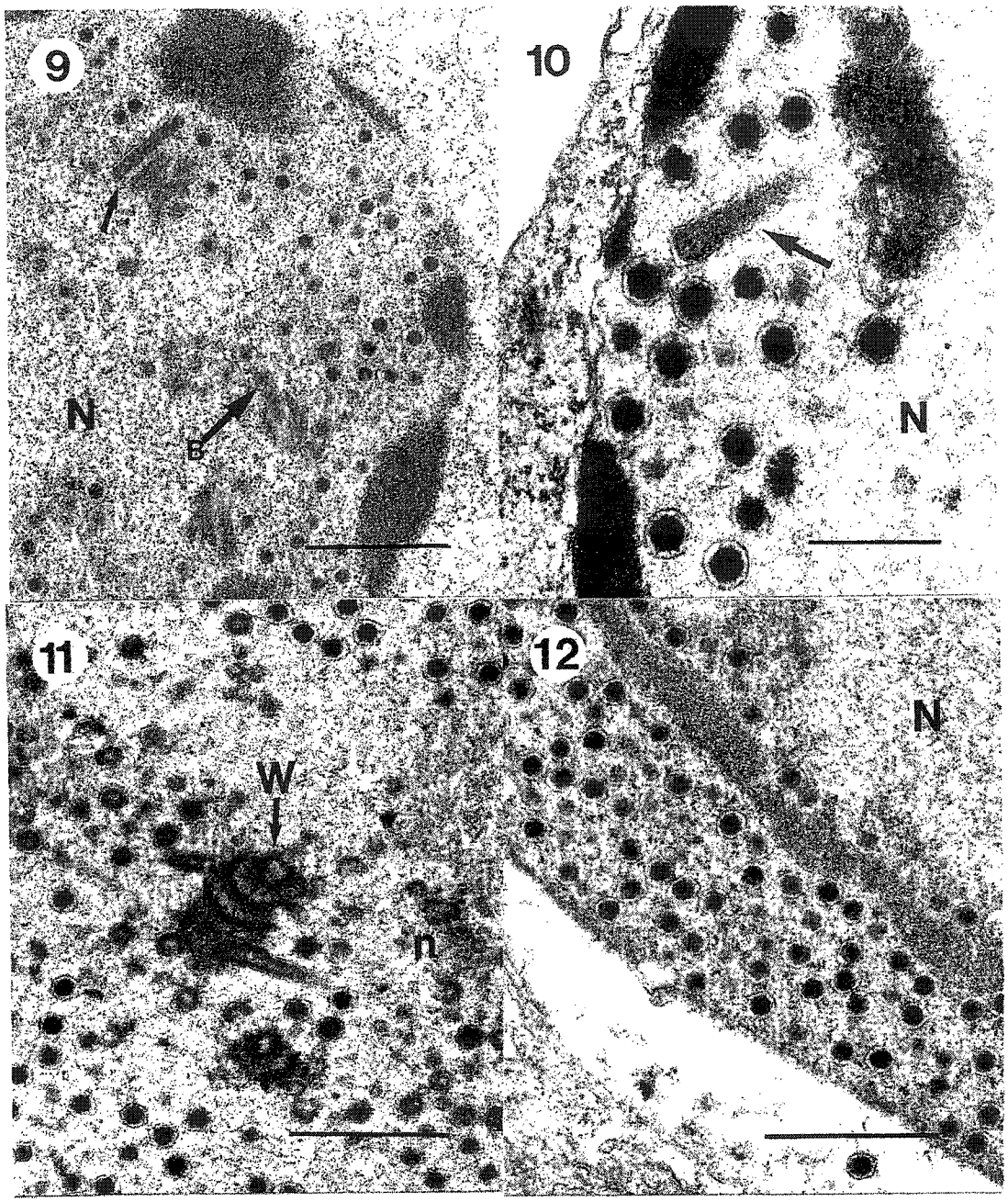

FIG. 9. Nucleus (N) of infected hemocyte showing rod-like viral assembly sites (arrows) with a nucleocapsid possibly budding off (B) from one of the rod-like elements. Bar = 1 μm.

FIG. 10. Detail of a rod-like element showing the coalescence of the capsid along a portion of the rod which is arising from the granular matrix. Bar = 500 nm.

FIG. 11. Rod-like assembly site shown arising from an intranuclear whorl (W) of electron dense material. Note the presence of toroids, nucleoids without capsids and the finely granular nuclear matrix. Bar = 1 μm.

FIG. 12. Loose aggregate of virions in the cytoplasm adjacent to hypertrophied host nucleus (N). Bar = 1 μm.

Discussion

In collaboration with Dr. Jeffrey Shields (Virginia Institute of Marine Science), I have discovered and identified the first naturally occurring pathogenic virus from a lobster. The agent is an unenveloped, nonoccluded, herpes-like DNA virus (HLV-PA) with a predilection for the hemocytes and connective tissue cells of juvenile lobsters. HLV-PA is unlike members of the Herpesviridae in that the virions are unenveloped and there are no obvious inclusion bodies present in the nuclei of infected cells. However, the assembly within the host nucleus, the electron-lucent cylindrical core surrounded by an electron-dense toroid and the icosahedral bilayered capsid are morphological features shared with the Herpesviridae.

Herpes-like viruses have been reported from three other crustaceans. A pathogenic herpes-like virus (bifacies virus) infected the hemocytes of blue crabs, *Callinectes sapidus*, and was transmissible via cannibalism and injection (Johnson 1976, 1983). It was pathogenic, killing inoculated hosts in 30 d, and reportedly prevalent in 13% of juvenile crabs (Johnson 1983). Bifacies virus was initially reported as a herpes-like virus (Johnson 1976), but upon better fixation it was shown to differ significantly in morphology from the Herpesviridae by possessing an envelope synthesized within the nucleus, no capsid and an electron-dense core region (Johnson 1988). A herpes-like virus was reported in the germinative testicular cells of the mud crab, *Rhithropanopeus harrisii*, but it was not known if the virus caused morbidity or mortality (Payen and Bonami 1979). A pathogenic herpes-like virus was found in the bladder and antennal gland epithelia of Alaskan king crabs, *Paralithodes platypus, Paralithodes camtschaticus,* and *Lithodes aequispina* (Sparks and Morado 1986). The virus was found

at relatively high prevalences of 15% to 17% and was thought to have contributed to major declines in the red king crab fishery in 1982-83. The hexagonal virus was unenveloped in the nucleus of the host cell but virions were not visualized outside the infected cell (Sparks and Morado 1986). Further, large, irregular nuclear inclusion bodies were present in host cells infected with the herpes-like virus, a condition not observed in HLV-PA.

Naturally occurring viral infections have not been reported from lobsters. However, the host range and pathology of an important shrimp virus, white spot virus (WSV), has been examined in experimentally infected spiny lobsters. Using a DNA probe specific to WSV, Chang et al. (1998) detected the virus in the gills, stomach, cuticular epidermis and hepatopancreas of *Panulirus versicolor* and *Panulirus penicillatus.* They did not assess the pathological consequences of infection nor the potential for transmission. Wang et al. (1998) used PCR to detect WSV in *P. versicolor*, *P. penicillatus*, *P. ornatus* and *P. longipes* that had been experimentally infected through ingestion of infected shrimp. Although all of the exposed lobsters survived, WSV was detectable in their tissues at low levels. Another experimental study has shown that WSV has a wide host range in several other decapods (Supamattaya et al. 1998).

We speculate that viral assembly of HLV-PA occurs along the rod-like structures present in the virogenic stroma. The nucleoids apparently coalesce along these rods and capsid elements clearly occur there. Small fibrillar rods or strands have been reported as intranuclear inclusions in cytomegalovirus (CMV) infections (Cavallo et al. 1981) and in herpes-like infections in the flat oyster (Hine and Thorne 1997). In CMV, the rods, and the granular nuclear matrix, are the sites of viral DNA synthesis with viral assembly

occurring along the edges of the matrix (Fong 1982, Wolber et al. 1988). Unlike the Herpesviridae, morphogenesis of the virions of bifaces virus of blue crabs initiates with the formation of a region of the outer and inner envelopes followed by successive condensations of the toroid and core regions prior to completion of the envelope (Johnson 1988). Thus, HLV-PA is morphologically more like a herpes virus than the bifacies virus.

The pathology of infected spiny lobsters shows a marked depletion of reserve inclusions (RIs) in cells of the spongy connective tissues. Glycogen is one of the main storage products in the RIs (Travis 1955, Johnson 1980); it is the substrate for several physiological processes including energy storage and chitin synthesis (e.g., Heath & Barnes 1970, Stevenson 1985). Glycogen depletion may be a common pathological consequence of microbial infections in decapods (Stewart and Arie, 1973; Shields et al., in press). The loss of RIs with the commensurate loss in glycogen indicates that the energy storage of infected individuals is compromised and that metabolic exhaustion coupled with ischemia from anaerobic metabolism is a likely cause of death.

It is possible that current fishing practices may accelerate the transmission and spread of this disease. Commercial fishermen use live juvenile lobsters in traps as "bait" (i.e., a social attractant) for larger adults. The close proximity of lobsters confined in traps and the confinement of juveniles by the hundreds in live-wells, along with the physiological stresses induced by such practices, could facilitate the spread of infectious diseases. Transport of juvenile lobsters throughout the fishing grounds could also facilitate the spread of pathogens. Given the notoriety of viral infections in shrimp (for review, see Brock and Lightner 1990), the pathology of similar infections in blue and

king crabs, and my initial data, I believe that further characterization of this virus is warranted.

A greater understanding of this virus is important not only for the obvious stability of this important fishery and the untold number of livelihoods it supports, but also because HLV-PA appears to alter the behavior of the juvenile lobsters it infects in remarkable ways (see Chapter III). These behavioral alterations extend beyond those typically observed in infected individuals and indirectly alter the behavior of healthy lobsters in relation to diseased conspecifics through recognition of infection; a consequence rarely seen among animals, aquatic or terrestrial, vertebrate or invertebrate. Further, investigating the mode(s) by which the virus is transmitted is essential to understanding how the virus is spread, the role the fishery may play in the spread of the virus, and how these behavioral alterations may have arisen (see Chapter III). This information is, in turn, complimented by an understanding of how the characteristics of the lobster population (e.g., nutritional condition and density) affect disease prevalence (see Chapter IV).

CHAPTER III

DISEASE IN JUVENILE CARIBBEAN SPINY LOBSTER POPULATIONS: BEHAVIORAL ALTERATIONS AND MODES OF TRANSMISSION

Introduction

Diseases and parasitic infections can increase host mortality both by directly affecting the physiological condition of the host, or by influencing host susceptibility to predation. Impaired predator detection abilities (Lefcort and Blaustein 1995), altered avoidance behavior (Moore 1983, Lefcort and Eiger 1993, McCarthy et al. 2000) reduced or eliminated flight responses (Giles 1983, Godin and Sproul 1988), and increased conspicuousness (Carney 1969) are all examples of how disease can indirectly alter the risk of predation to their host (see Holmes and Bethel 1972, Dobson 1988, Poulin 1995 for reviews). Studies abound that demonstrate the adaptive significance of increased host vulnerability to pathogens, especially for those requiring multiple hosts for completion of their life-cycle (Brassard et al. 1982, Barnard and Behnke 1990, Ewald 1994, Moore 1995, Lefcort and Blaustein 1995). Whether the behavioral aberration to hosts brought on by infection is an adaptation that serves to expedite transfer of a parasite to another host, or is simply a by-product of infection can be difficult to distinguish (Moore and Gotelli 1990, see Poulin 1995 for review).

Although there are many extraordinary examples of parasite-mediated changes in host behavior, the behavioral response of hosts subject to bacterial or viral disease is often non-specific, including depression and lethargy. In rare instances, behavioral modification by bacterial or viral infections can be spectacular, as is the case with the virus that causes rabies in mammals (MacDonald 1980, Hart 1988, 1990). Canids and mustelids infected with this virus turn vicious and wander, randomly attacking and biting other mammals they encounter. The virus, which cannot survive outside of a living mammal, is thus transferred to a new host via the salivary secretion of the infected individual. More commonly, host activity decreases in response to bacterial or viral infection, which is not likely to increase transmission of the pathogen in the host population. In fact, it is likely to act in just the opposite manner by reducing contact among hosts (Loehle 1995) and allowing the host to preserve energetic resources and resist or eliminate the pathogen (see Hart 1988 for review). Active avoidance of diseased individuals by healthy conspecifics would, theoretically, be selectively advantageous for the host if disease transmission is dependent on contact among potential hosts (Loehle 1995, Kiesecker et al. 1999), but remarkably few organisms have evolved such adaptations.

Except for several studies of the habituation period that primate groups impose on newcomers, a behavior hypothesized to reduce the introduction of foreign parasites (Freeland 1976), investigations of the impact of diseased conspecifics on the behaviors of uninfected individuals are limited primarily to studies of the role of diseases and parasites in altering mating systems. For example, based on their studies of passerine birds infected with several blood parasites, Hamilton and Zuk (1982) developed the theory that

more impressive behavioral displays signify a mate with greater genetic parasite resistance because these displays are linked to greater health and vigor. Kennedy et al. (1987) used the same theory to demonstrate the influence that two parasites, a gut dwelling nematode (*Camallanus cotti*) and an ectoparasitic monogenean (*Gyrodactylus sp.*), have on mate selection in guppies (*Poecilia reticulata*). Female guppies preferentially select males that engage in a greater rate of display, which is in turn associated with higher resistance to pathogens (Lopez 1998). Female mice (*Mus musculus*) not only detect a difference between infected and healthy male mice, preferring the latter, but they also elicit a more acute response to danger when exposed to the odiferous secretions of males infected with a nematode parasite, *Heligmosomoides polygyrus* (Kavaliers and Colwell 1995, Penn and Potts 1998). Although these studies all indicate that avoidance of diseased mates benefits the reproductive success of healthy individuals, the benefits of social disassociation in reducing infection risk to healthy mates is unknown.

Evidence for the avoidance of diseased individuals by healthy conspecifics, outside the realm of mating interactions, is non-existent except for a single study on tadpoles. Kiesecker et al. (1999) recently discovered that healthy bullfrog tadpoles (*Rana catesbeiana*) avoid conspecifics infected with an intestinal yeast parasite (*Candida humicola*). The potential benefit to healthy individuals capable of quickly assessing the status of conspecifics infected with transmissible diseases is obvious. The only caveat being that the mode of transmission must be such that avoidance is an effective means of reducing infection risk (Loehle 1995). However, biologists often conclude that these advantages are countered by the advantages imparted by sociality (e.g., resource

detection, reproduction efficiency, and predation avoidance) and thus the presence of infected conspecifics is viewed only as a cost to social behavior (Loehle 1995).

If the ability to detect and avoid infected conspecifics is to reduce infection risk, transmission must be possible through mere proximity to an infected animal. Detection and avoidance would not be as advantageous if, for example, the infectious agent were mobile and could seek out the host or was acquired through ingestion of an intermediate host. Pathogens in marine systems may be transmitted by ingestion of infected material, waterborne transport of pathogens or physical contact with an infected host. The specific site of entry or site of attachment is often very specific to the pathogen-host relationship. Oral ingestion of tissues infected by the pathogen (e.g., detritus or an infected prey item) and its subsequent invasion through the intestinal wall is one of the most common modes of infection (Bang 1983). Waterborne- or contact-transmitted pathogens may also gain access to a host through the skin or cuticle. One of the earliest studies of comparative pathology by Metchnikov (1892) involved infection of *Daphnia* directly through the cuticle (van Uden and Castelo-Brancho 1961) by the parasitic yeasts, *Metschnikowiella zobelli* and *M. krissi*. Other pathogens invade the host through soft, external tissues. White spot syndrome virus (WSSV) is infectious if hosts are submersed in a suspension of virus particles (Supamattaya et al. 1998, Chen et al. 2000). Progressive analysis of tissues of the mud crab (*Scylla serrata*) following exposure showed initial infection of the gills and integument followed by later infection of the internal organs (Chen et al. 2000). Other diseases such as gaffkemia, a bacterial disease that infects clawed lobsters and caused by the bacterium *Aerococcus viridans*, are transmitted through injuries to the cuticle, cuticular membranes, or autotomized limbs (for review see: Shapiro et al. 1974,

Stewart 1980, Evans and Brock 1994). For gregarious marine animals such as spiny lobster, that dwell together in communal dens, all of the above modes of transmission are possible. In fact, pathogens often have a higher prevalence and infection intensity within social species (see Cote and Poulin 1995 for review).

All organisms maintain defenses to the invasion of pathogens. These include both physical barriers such as the skin or exoskeleton and internal defenses such as humoral and autoimmune responses, phagocytosis, lysis, and infiltration. For crustaceans, humoral defenses include circulating bactericidins, agglutinins, opsonins, lysins and precipitins, phagocitic and encapsulating activity of hemocytes, and infiltration by hemocytes to a cite of injury or infection (see Evans and Brock 1994 for review). These defenses, though normally formidable, may become compromised in the presence of physiological or environmental stresses (Brock and Lightner 1990). Stress can act to weaken the immune system allowing invasion and infection by external pathogens or multiplication of opportunistic disease causing organisms. For example, when oysters (*Crassostrea gigas*) are stressed via mechanical disturbance their immune response, as measured by the stress indicators noradrenaline and dopamine, is initially compromised but then rebounds shortly after the period of stress (Lacoste et al. 2002). Environmental stress, particularly temperature and salinity, can negatively affect the growth and survival of early benthic stage spiny lobsters, *Panulirus argus*, (Field and Butler 1994) as well as the movement and respiratory efficiency of clawed lobsters, *Homarus americanus*, (Jury et al. 1994*b*, Jury and Watson 2000), and can affect the time course and susceptibility of lobsters to disease (Stewart 1980). This heightened susceptibility in the face of environmental change is not unique to lobsters, occurring in invertebrates and vertebrates

alike. Rogers and Burke (1981) reported a substantial increase in "red spot" disease, attributed to the bacterium *Vibrio anguillarum*, in the sea mullet, *Mugil cephalus* following periods of heavy rainfall when salinity dropped appreciably. Lafferty and Kuris (1999) review numerous environmental stresses that have been shown to increase the effects of parasites including pollutants (e.g., oil and industrial effluents), habitat alterations, fishing pressure, and introduced species.

Diseases in lobsters are few (see Chapter II and Evans et al. 2000 for review), but notably absent are viral pathogens. No naturally occurring viruses have been previously identified in lobsters, but lobsters have been experimentally infected with viruses. A highly pathogenic virus found in shrimp, white spot sydrome virus (WSSV), which decimated shrimp aquaculture farms in Asia and Central America in the 1990s, has been experimentally transferred to several spiny lobster species. Chang et al. (1998) used a DNA probe to detect WSSV in selected tissues of *P. versicolor* and *P. penicillatus*, which they experimentally infected via direct inoculation with infected hemolymph. Similarly, Wang et al. (1998) experimentally infected *P. versicolor*, *P. penicillatus*, *P. ornatus* and *P. longipes* through ingestion of infected shrimp (*Penaeus monodon*) tissue. However, this virus does not occur naturally in lobster populations.

A Viral Disease in Caribbean Spiny Lobster

While sampling juvenile Caribbean spiny lobster (*P. argus*) populations in the Florida Keys during the summer of 1999, I discovered what we now know to be the first documentation of a pathogenic viral disease in a lobster species (Shields and Behringer *in press*). The gross symptoms of this abnormality are lethargy, morbidity, a cessation of molting, fouling of the carapace, and most notably, discoloration of the hemolymph.

Lobster hemolymph is normally clear with a grayish-blue or amber tint, depending on molt stage, but when infected with the virus the hemolymph is milky or chalky white presumably due to the large amount of cellular debris present. The lethargy observed in late-stage infections may be due to their depressed nutritional condition and diminished glycogen reserves, perhaps as a result of their reduced feeding. In heavily infected individuals, virtually all of the hyalinocytes and semigranulocytes of the host are destroyed. Histopathology of tissues from infected lobsters and transmission electron microscopy confirmed the disease to be an non-enveloped, icosahedral, Herpes-like DNA virus (HLV-PA) (Shields and Behringer *in press*).

In this chapter, I examine: (i) the spatio-temporal patterns of the prevalence of the viral disease among juvenile lobster populations in the Florida Keys, (ii) the potential means by which the virus is transmitted among lobsters, (iii) the impact of the disease on the local movement and social behavior of diseased and healthy lobsters, and (iv) the effect of HLV-PA infection on the nutritional condition of juvenile lobsters.

Background

Spiny lobsters undergo an ontogenetic change in social behavior. They are asocial as early benthic juveniles but become highly gregarious, social creatures as late-stage juveniles and adults (Berrill 1975, Eggleston and Lipcius 1992, Childress and Herrnkind 1994, 1996, Ratchford and Eggleston 1998, 2000), a strategy that enhances the survival of some species (Butler et al. 1997, 1999). Caribbean spiny lobsters begin their benthic existence following a 9 - 12 month (Lewis et al. 1952, Kittaka 1994) larval period in the oceanic plankton. In Florida, they enter shallow nursery grounds in a transitional post-larval (puerulus) form each new moon and settle into the ubiquitous red macroalgae,

Laurencia spp., found in hard-bottom habitats throughout the Florida Keys. They remain hidden, camouflaged, and solitary in the macroalgae for several months (Marx and Herrnkind 1985*b*) until they reach 15 – 20 mm carapace length (CL), when they emerge from the macroalgae (Marx and Herrnkind 1985*a*), become gregarious, and seek out crevice shelters as a daytime refuge, often dwelling together (Eggleston and Lipcius 1992, Childress & Herrnkind 1994, 1996). As they approach maturity, approximately 18 – 24 months after settlement, *P. argus* migrate from shallow coastal nurseries to fringing or barrier reefs where the adults dwell. Thus, juveniles and adults live in separate habitats separated by tens of kilometers.

Methods

Field Procedures

A. Assessing Disease Prevalence and Distribution:

Field surveys to assess the prevalence and distribution of the HLV-PA virus in juvenile spiny lobsters were conducted in the summer of 2000, winter of 2001, and the summer of 2001 at 12 sites (9 in summer 2000) on the bayside of the middle and lower Florida Keys, USA (Fig. 13). Site locations were chosen haphazardly within the hard-bottom nursery areas available in the region. Hard-bottom habitat is found throughout the shallow waters surrounding the Florida Keys and comprises 30 – 40% of the available bottom < 3 m deep (Zieman et al. 1989, Herrnkind et al. 1997*b*). Each site was a 625 m^2 area delineated with polypropelene rope attached at four corners to concrete blocks. Sites ranged in depth from 1 - 3 m and were approximately 100 m to 7 km from shore.

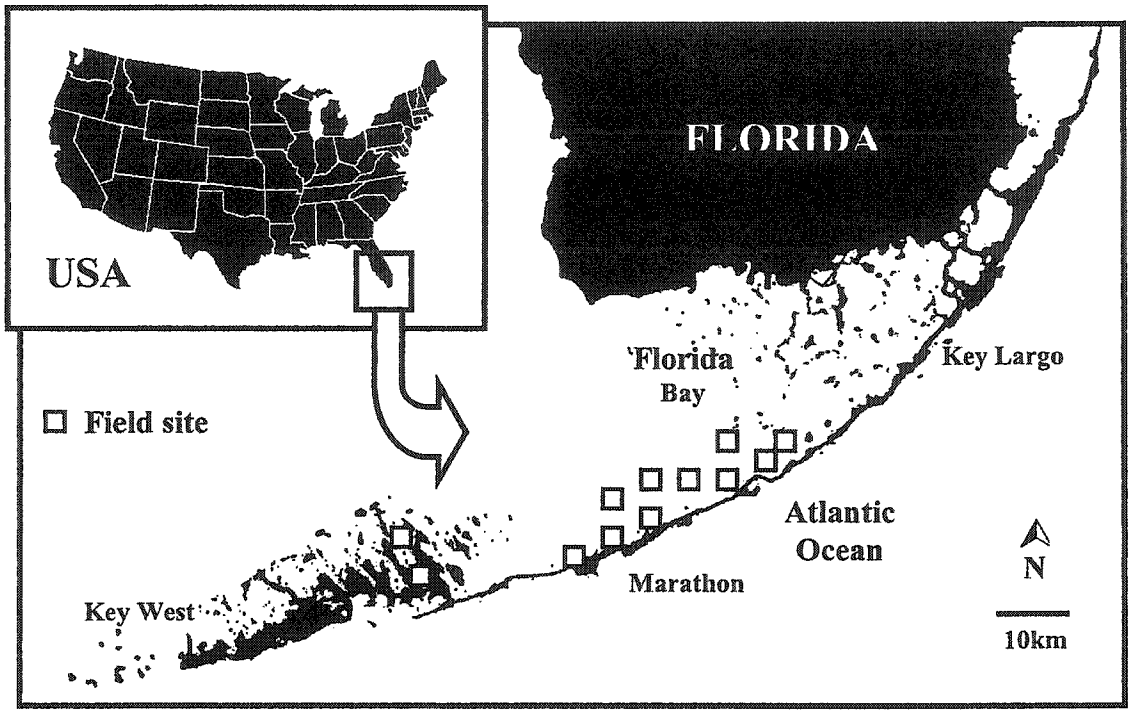

FIG. 13. Location of the field sites in the Florida Keys (USA) wherein disease prevalance was monitored and where field observations of sociality and den fidelity were made.

B. Impact of Disease on Lobster Short-term Residency:

To assess the impact of the disease on the short-term residency of lobsters at field sites, I performed mark-recapture studies over a five-day period during each survey. On the initial day of the mark-recapture period, one or two divers searched the entire site, captured each lobster, and recorded the carapace length (CL), sex, molt condition (pre-molt, inter-molt, or post-molt), and the health status (diseased or healthy) of each individual collected. The lobster was then marked with a unique color-banded antenna tag and returned to its den. Five days later, the divers searched the entire site again, captured all lobsters encountered, and recorded the tag code (if present, otherwise the lobster was measured and sexed for later identification) and the number of conspecifics with which it was found cohabiting in a den. The lobsters were then brought on-board

the research vessel where the presence or absence of visible disease was recorded. Discolored (i.e., chalky or milky white) hemolymph was used as evidence of infection and was viewed via inspection of the dorsal juncture between the cephalothorax and the abdomen.

C. Impact of Disease on Lobster Social Behavior:

The potential difference in co-occupancy of dens between healthy and diseased juvenile lobsters was assessed on the final day of the mark-recapture. While underwater I recorded the presence and number of conspecifics co-occupying dens, and then onboard the research vessel I assessed all lobsters for the visible presence of HLV-PA infection.

D. Impact of HLV-PA infection on nutritional condition:

The impact of HLV-PA infection on nutritional condition was also assessed on the final day of the mark-recapture period. When lobsters were brought on board the research vessel, a subset of 20 lobsters was sampled to ascertain their hemolymph refractive index (see Chapter IV for a full description). In short, I used a 25-gauge tuberculin syringe to draw 0.1 ml of hemolymph from the proximal joint of the 5^{th} periopod and deposited it onto a Leica industrial refractometer, read to the nearest 0.5 units.

Laboratory Procedures

Impact of Disease on Lobster Social behavior:

My field observations suggested that diseased lobsters were alone in shelters more often than healthy lobsters. Therefore, in a series of laboratory mesocosm experiments, I

tested whether healthy lobsters actively avoided diseased conspecifics or if diseased individuals isolated themselves (Fig. 14). The mesocosm studies were performed in the spring and summer 2001 at the Florida Fish and Wildlife Conservation Commission – Florida Marine Research Institute (FWC) laboratory in Marathon, Florida and the Keys Marine Laboratory in Layton, Florida. Lobsters for these experiments were collected from the near-shore bayside waters of the Florida Keys. The mesocosms were round tanks approximately 2.0 m wide and 1.0 m deep and were supplied with flow-through ambient temperature seawater. In each mesocosm I placed two dens, each composed of a 20-cm long x 10-cm diameter PVC cylinder attached to a fragment of cinder block to stabilize the den. I tethered a lobster (either diseased or healthy) in one PVC den and left the other den open. Tethering was accomplished by attaching a fishing swivel to a small cable-tie and attaching this to the distal portion of the abdomen immediately before the uropods. A 20 cm piece of monofilament fishing line was used to attach the swivel to the PVC den. An un-tethered focal lobster was then introduced into the mesocosm and allowed 24 hrs to choose a den, at which time I recorded the location of the focal lobster. Four treatment combinations were tested: (1) healthy focal lobster with a diseased tethered lobster, (2) healthy focal lobster with a healthy tethered lobster, (3) infected focal lobster with a healthy tethered lobster, and (4) infected focal lobster with an infected tethered lobster.

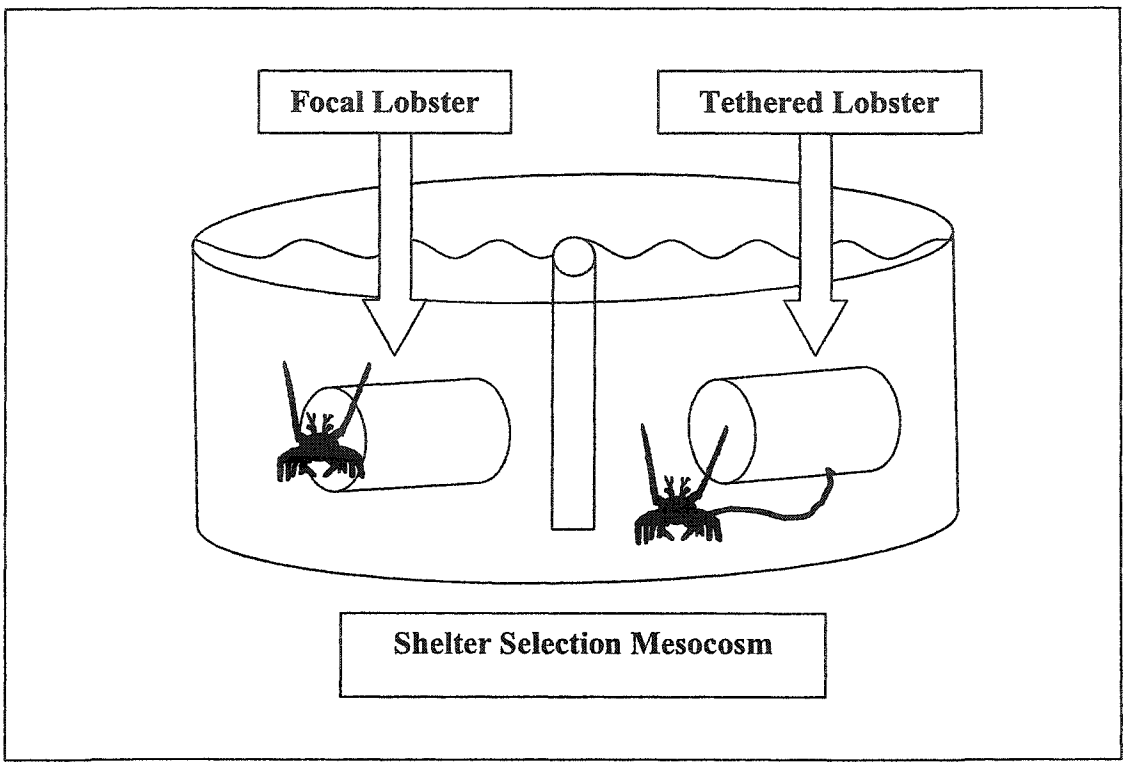

FIG. 14. Diagram of laboratory mesocosm set-up designed to test for the impact of disease on den co-occupancy patterns. The mesocosm tank was a 2 m diameter x 1 m deep tank equipped with flow-through ambient seawater. On opposite sides of each mesocosm I placed two anchored PVC pipe shelters. To one of the shelters a lobster, either diseased or healthy, was tethered; the other shelter remained open. An un-tethered focal lobster (either diseased or healthy) was introduced and allowed 24 hrs to acclimate.

Transmission

A. Disease Transmission by Inoculation

To confirm the transmissibility of the HLV-PA virus, 21 juvenile lobsters captured from the field were inoculated with hemolymph from an infected individual and maintained in isolation for 80 days in flow-through seawater tanks at the FWC laboratory. Un-inoculated individuals (n = 5) were also held under identical conditions to serve as controls. These data were initially reported in Shields and Behringer (*in press*). Lobsters were captured from hard-bottom habitat at a bayside site in the Florida Keys by

divers using hand nets, and then transported to the laboratory for processing. There, I recorded the CL, sex, molt stage, and total hemolymph (blood) protein concentration for each individual (see Chapter IV for a description of the hemolymph protein method). Molt stage was determined from microscopic examination of pleopod samples as described by Lyle and MacDonald (1983). I inoculated lobsters using a 1-cc tuberculin syringe with a 27-ga. needle containing either 0.1 ml (n = 10 lobsters) or 0.2 ml (n = 11 lobsters) of raw infected hemolymph. I injected the infected hemolymph through an alcohol sterilized arthrodial membrane at the juncture of the basis and ischium of the 5th walking leg. Lobsters were then maintained in the laboratory in individual containers and monitored for up to 80 days, if they survived that long. During this period, they were fed frozen shrimp and squid *ad libitum* every two days. I used light microscopy of hemolymph smears taken every 10 -14 days to follow the progression of the disease in inoculated lobsters. Smears were made by placing 1 - 2 drops of hemolymph on a poly-L-lysine coated slide, which I air dried, and then fixed in 100% methanol for 45 s. Upon termination of the experiment after 80 days, I obtained a final hemolymph smear for each surviving lobster and then obtained tissue samples (heart, gills, hepatopancreas, abdominal muscle, foregut and hindgut) from cold-anesthetized lobsters. The tissue samples were fixed in 10% neutral buffered formalin and preserved in 70% ethanol for histological examination via light microscopy by Dr. Jeffrey Shields at the Virginia Institute of Marine Science.

B. Disease Transmission by Ingestion and the Impact of Stress on Transmission

Ingestion and subsequent invasion of pathogens through the intestine wall is one of the most direct means of disease transmission (Bang 1983). Recent studies of the transmission of WSSV have shown that ingestion is an effective means of transmission of this virus to a wide variety of other crustaceans including: copepods, the mud crab *Scylla serrata*, the sand crab *Portunus pelagicus*, the krill *Acetes* sp. (Supamattaya et al. 1998) and numerous marine and freshwater shrimp (Wang et al. 1998).

Thus, to investigate whether lobsters are subject to food-borne transmission of the HLV-PA disease, I carried out an experiment in which 28 lobsters were held in isolated flow-through seawater tanks and fed abdominal muscle tissue from infected conspecifics. Concurrent with this investigation, I also explored the impact of physiological stress on disease transmission by ingestion. As noted earlier, portions of Florida Bay where juvenile lobster nurseries occur are also exposed to differing salinities due to changes in climatic conditions and land-use patterns in the Everglades (Boyer et al. 1997, Nuttle et al. 2000). Salinities that deviate from those typical of open ocean conditions (i.e., < 35 psu or > 35 psu) are stressful to clawed (Jury et al. 1994) and spiny lobsters (Field and Butler 1994). I therefore exposed the inoculated lobsters to one of four different salinity regimes: 15 psu, 25 psu, ambient seawater (36 - 39 psu) and 45 psu. Lobsters were starved for 10 d prior to the initiation of the experiment to ensure ingestion of infected tissue. Then once a week for four weeks, I fed them approximately 1 g of abdominal muscle tissue from an infected lobster. Lobsters were fed frozen shrimp and squid every other day at all other times. Two additional lobsters were held in each of the four salinity treatments and fed a diet of squid and shrimp *ad libitum* to serve as controls.

I took a hemolymph sample from each individual every 14 d and preserved the samples (10 parts 10% neutral buffered formalin: 1 part raw hemolymph) from each lobster for later determination of the initiation of infection. Hemolymph and tissue samples were collected and preserved (see above) from all lobsters that appeared moribund or displayed advanced stages of the virus during the course of the experiment. Upon termination of the experiment after 80 days, I obtained hemolymph and tissue samples (see above) from each surviving lobster for histological examination by Dr. Jeffrey Shields at the Virginia Institute of Marine Science.

C. Disease Transmission by Contact or Waterborne Means

To investigate the infection of visibly healthy lobsters when cohabitating with diseased lobsters, I maintained three non-infected juvenile lobsters of different sizes with a diseased individual in each of 10 isolated, flow-through seawater tanks. For controls, an additional five tanks were maintained with three healthy lobsters each. Note that control lobsters were visually inspected and presumed non-infected, but only at the termination of the experiment when all lobsters were histologically examined was their actual disease status determined. To test whether smaller lobsters were more susceptible to the virus than larger ones as my field observations suggested, the three healthy lobsters held in each tank were of different size classes (small 20 - 30 mm, medium 30 - 40 mm, and large 40 - 50 mm CL, comprising a randomized-block design). Lobsters were fed *ad libitum* a diet of shrimp and squid. Prior to initiation of the experiment, lobsters were measured (CL), their sex determined, injuries noted, nutritional condition measured (i.e., hemolymph refractive index, see Chapter IV), and molt stage determined. I also obtained and preserved (see above) a hemolymph sample from each experimental lobster to

ascertain whether the HLV-PA disease was present in any individual prior to initiation of the experiment. Hemolymph was drawn and preserved (10 parts 10% neutral buffered formalin: 1 part raw hemolymph) from each animal every 14 days until such time that they became moribund, died or displayed advanced stages of the disease. Upon termination of the experiment after 80 d, a hemolymph smear was made for each surviving lobster. Lobsters were subsequently sacrificed and selected tissues (heart, gills, hepatopancreas, abdominal muscle, foregut and hindgut) fixed in 10% neutral buffered formalin for 48 hrs and then preserved in 70% ethanol for histological examination at the Virginia Institute of Marine Science, by Dr. Jeffrey Shields.

Statistical Analyses

Observations made during my field surveys of disease prevalence suggested that most of the visible HLV-PA infections I documented were from small juveniles. Therefore, I used a 4 x 3 model I repeated-measures analysis of variance (ANOVA) to examine differences in HLV-PA prevalence with lobster size. The factor of interest was lobster size, which consisted of four size-specific treatment groups: < 20 mm CL, 20 - 30 mm CL, 30 - 40 mm CL and > 40 mm CL. These groupings reflected the maximum number of size groups in which there was sufficient replication in each group for the analysis, and also conformed to sizes at which ontogenetic changes in behavior have been noted (Berrill 1975, Smith and Herrnkind 1992, Ratchford and Eggleston 1998, Childress and Herrnkind 1994, 1996, 2001). The seasonal surveys were conducted at the same field sites, so the second repeated-measures factor was survey period, which had three levels: June-August 2000, January-March 2001 and June-August 2001. These data were

arcsine transformed to meet the ANOVA assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test).

To determine if cohabitation among lobsters in the field was independent of disease or survey period, I used a three-way contingency table analysis. The first factor, survey, had 3 levels representing the three survey periods: June-August 2000, January-March 2001 and June-August 2001. The second factor, lobster health status, had two levels: visibly infected and visibly non-infected. The third factor, cohabitation, had two levels: solitary or cohabitating.

The impact of HLV-PA on short-term residency rates was similarly evaluated using a three-way contingency table analysis. The same survey and health status factors were used, but the third factor was recapture history i.e., whether a lobster was recaptured or not recaptured after the five-day mark-recapture.

A 2 x 4 model I repeated-measures ANOVA was used to determine if HLV-PA infection had any impact on the mean hemolymph refractive index of visibly infected lobsters as opposed to visibly healthy lobsters. The first factor in this analysis was health status (visibly infected and visibly healthy) and the second factor was sampling date. A Shapiro-Wilk test and a Levene's test were used on the data to test normality and homogeneity of variances assumptions. The mean hemolymph refractive index data were both normally distributed and the variances were homogeneous among levels, so the raw data were used in the analysis. Though hemolymph refractive index has been demonstrated to vary with molt stage (see Chapter IV), it was not included as a covariate in this analysis because lobsters infected with HLV-PA are exclusively in the intermolt stage.

The data from the shelter-choice experiments, wherein I investigated the impact of HLV-PA on lobster social behavior, was evaluated using a 4 x 2 contingency table analysis. The first factor, treatment, had four levels: (1) healthy focal lobster with a diseased tethered lobster, (2) healthy focal lobster with a healthy tethered lobster, (3) infected focal lobster with a healthy tethered lobster, and (4) infected focal lobster with an infected tethered lobster. The second factor, cohabitation outcome, had two levels: cohabitating and solitary.

The transmission experiment in which I tested the impact of salinity stress on oral ingestion transmission of HLV-PA was also evaluated using a 4 x 2 contingency table analysis. The first factor, salinity, had four levels: 15 psu, 25 psu, ambient (36-39 psu), and 45 psu. The second factor, infection status outcome, had two levels: infected and non-infected. For this experiment, the final infection status of each lobster was determined via histological examination and therefore represents the true infection status (as opposed to visual-only diagnosis in field surveys).

The effect of the contact/waterborne transmission experiment on each lobster were scored as ranks (0 – 3) based on infection level determined from histological examination of tissue samples: non-infected = 0, lightly infected = 1, moderately infected = 2, and heavily infected = 3. These ranks were then used in a 1-factor randomized block ANOVA. The factor of interest was lobster size, which had three levels: small (20 – 30 mm CL), medium (30 – 40 mm CL) and large (40 – 50 mm CL). The blocks in this analysis were the nine replicate experimental tanks in which one lobster from each of the three size groups was housed with a diseased lobster.

Results

Field Procedures

A. Assessing Disease Prevalence and Distribution:

The prevalence estimates reported here are based on visibly diagnosed infections (i.e., late-stage infections) and are therefore conservative. Field surveys at the 12 nursery sites in the middle and lower Florida Keys indicated that the virus was widespread in juvenile lobsters in this region, with a prevalence ranging from 0 - 37% per site. The overall prevalence was relatively consistent among the three surveys, ranging between 6.4 and 7.5% (Fig. 15). There was little difference in disease prevalence in lobsters sampled during the two summer and the one winter survey, but these surveys were of insufficient frequency to adequately assess seasonal patterns in disease prevalence.

Thus far, the disease appears limited to juvenile lobsters. The prevalence of infection differed significantly with lobster size class (i.e., < 20 mm CL, 20 – 30mm CL, 30 – 40 mm CL and > 40 mm CL) for data collected during the three field surveys (repeated measure) (Table 1). Only the smallest size class (0 – 20 mm CL) was significantly different from the other size classes (Fig. 16). In fact, 90% of all the infected lobsters were below 40 mm CL.

TABLE 1. A repeated-measures ANOVA testing for differences in the viral infection prevalence among size classes.

Source	df	Mean Square	*F*	*P*
Size class	3	0.319	5.367	0.002
Survey date	3	0.177	2.983	0.034
Error	130	0.059		

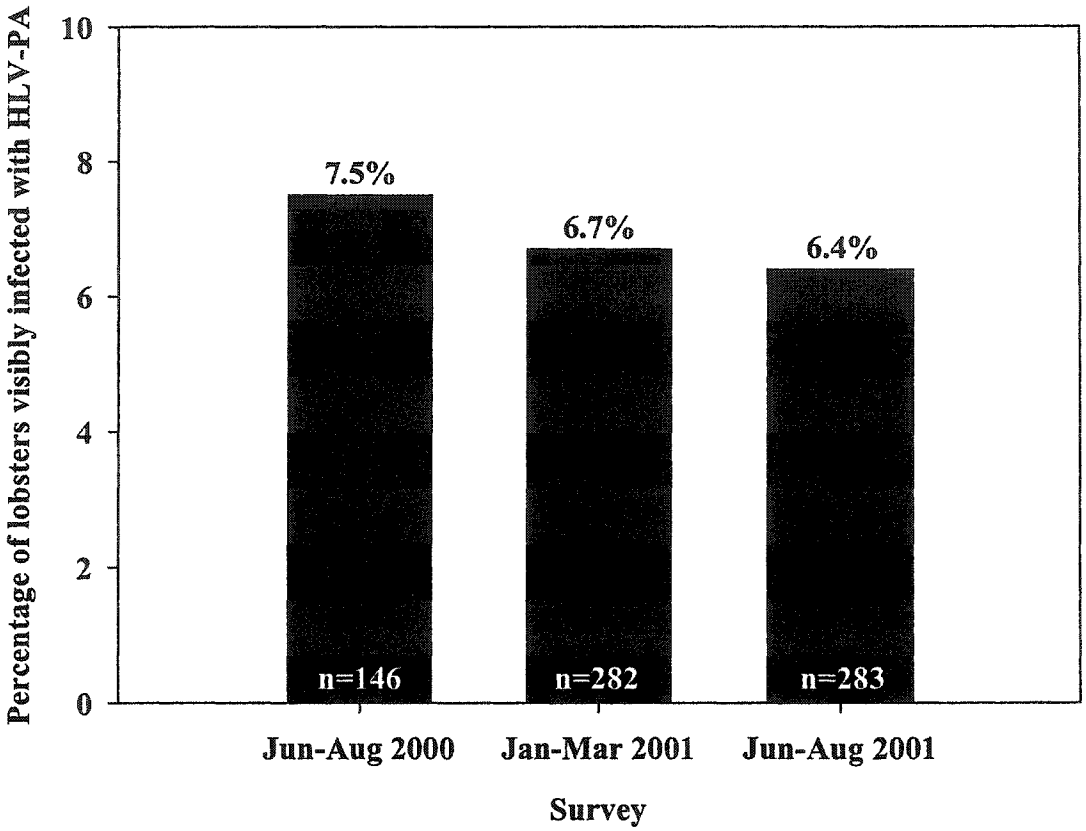

FIG. 15. Prevalence of HLV-PA infection in juvenile Caribbean spiny sampled at 9 – 12 sites in the middle and lower Florida Keys during three surveys in 2000-2001. Site locations did not change through successive surveys, although an additional three sites were included beginning with the winter 2001 survey.

B. Impact of Disease on Social Behavior:

The frequency of cohabitation among lobsters in the field was not independent of disease state or survey date (Table 2, Fig. 17). Lobsters infected with HLV-PA were significantly less likely to cohabitat with conspecifics than healthy lobsters, and this difference was consistent among surveys. Cohabitation status during the June–August 2000 survey alone differed from the January-March 2001 and June-August 2001 surveys (pair-wise contingency table analysis). Note that only three infected lobsters where found

cohabitating during all three surveys, and two of these where cohabitating with each other during the January-March 2001 survey.

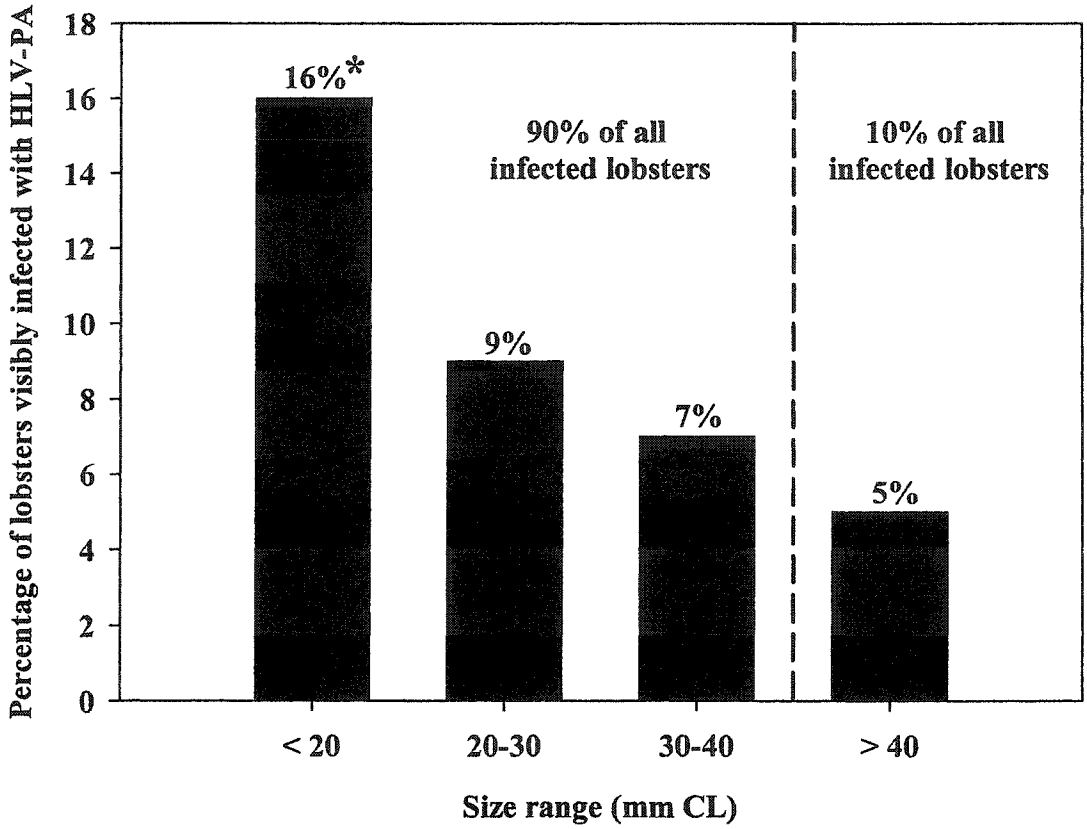

FIG. 16. Prevalence of infection in juvenile Caribbean spiny lobster by size class for the combined data from all three field surveys at sites in the middle and lower Florida Keys in 2000-2001 (*, $P < 0.01$).

TABLE 2. A three-way contingency table analysis of the difference in the frequency of shelter co-occupancy between visibly infected and visibly non-infected lobsters.

Interaction	df	Pearson X^2	P
Survey x Health status x Cohabitation status	2	0.420	0.8105
Survey x Health status	2	2.218	0.3298
Survey x Cohabitation status	2	14.395	<0.001*
Health status x Cohabitation status	1	57.238	<0.001*

*significance determined at alpha = 0.05

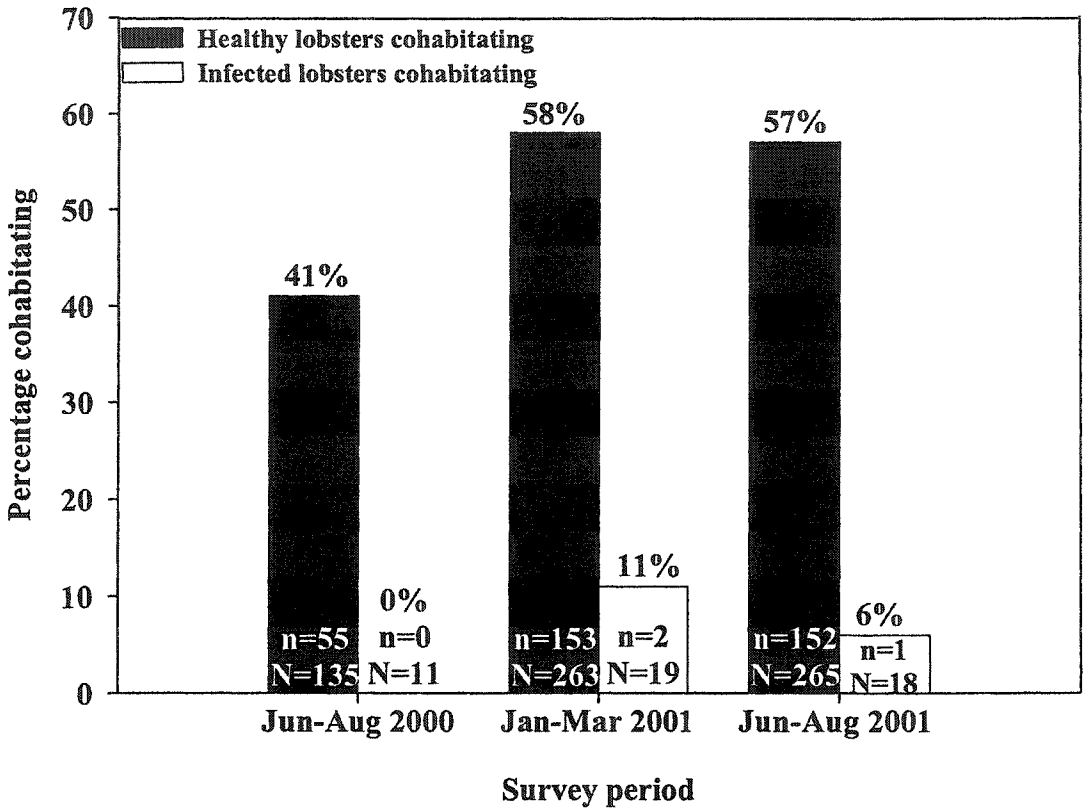

FIG. 17. Percentage of visibly healthy versus visibly infected juvenile lobsters found cohabitating in the same den during three field surveys in the middle and lower Florida Keys (n, number cohabitating; N, total number of lobsters).

C. Impact of Disease on Lobster Short-term Residency:

Analysis of the mark-recapture data indicated that there was no consistent difference in short-term residency rates between lobsters visibly infected with HLV-PA and lobsters not visibly infected. That is, recapture status was independent of health status when survey was not included (Table 3). The significant three-way interaction (survey x health status x recapture status) was due to a high recapture frequency of infected lobsters during the June-August 2000 survey, but is likely an artifact of the small sample size of infected lobsters (n = 11). However, this result supports my general

observation that infected lobsters are less active than their healthy conspecifics and perhaps more easily recaptured.

TABLE 3. A three-way contingency table analysis of the difference in the short-term residency between visibly infected and visibly non-infected lobsters.

Interaction	df	Pearson X^2	*P*
Survey x Health status x Recapture status	2	7.556	0.0229*
Survey x Health status	2	0.713	0.7000
Survey x Recapture status	2	2.159	0.3397
Health status x Recapture status	1	2.007	0.1565

* significance determined at alpha = 0.05

D. Impact of HLV-PA infection on nutritional condition:

The mean hemolymph refractive index of lobsters visibly infected with HLV-PA was significantly lower than that of visibly healthy lobsters (repeated-measures ANOVA; Table 4). The impact was similar among survey dates, with neither the survey date nor the interaction between health status and survey date significant in the analysis.

TABLE 4. A 2 x 4 model-I repeated-measures ANOVA examining the impact of HLV-PA infection on mean hemolymph refractive index.

Source	df	Mean Square	*F*	*P*
Health status	1	211.597	39.006	0.008*
Survey date	3	1.327	0.244	0.862
Health status * Survey	3	5.449	1.524	0.218
Error	59	3.576		

* significance determined at alpha = 0.05

Laboratory Procedures

Impact of Disease on Social behavior:

The laboratory cohabitation trials indicated that non-infected juvenile lobsters actively avoided cohabitation with diseased conspecifics (contingency table analysis, X^2 = 15.502, df = 3, P = 0.001; Fig. 18). In contrast, diseased lobsters chose to co-occupy shelters with other diseased lobsters just as frequently as with healthy lobsters. Note that in one of the only two cases where an infected lobster was found co-occupying a den with a conspecific in the field, that conspecific was also infected with HLV-PA. Moreover, the proportion of non-infected and infected lobsters found cohabiting in the laboratory trials (Fig. 18) closely resembled that seen in the field (Fig. 17).

Transmission

A. Disease Transmission by Inoculation:

In inoculation trials, 95% of the lobsters injected with hemolymph from HLV-PA-infected conspecifics became infected and 38% died within 30 – 80 days. All of the control lobsters injected with non-infected hemolymph survived, but one control lobster had developed an HLV-PA infection by the end of the trial. I am currently unable to detect HLV-PA in its early stages other than by histological examination of tissues, which requires dissection of the individual. Thus for experiments where lobsters could not be sacrificed for histological examination, I had to rely upon visual assessment of hemolymph color to discern healthy from infected individuals, which is only effective for lobsters in the late stages of the disease. Thus, the one control lobster that developed the disease had probably obtained the infection in the field prior to inclusion in the experiment.

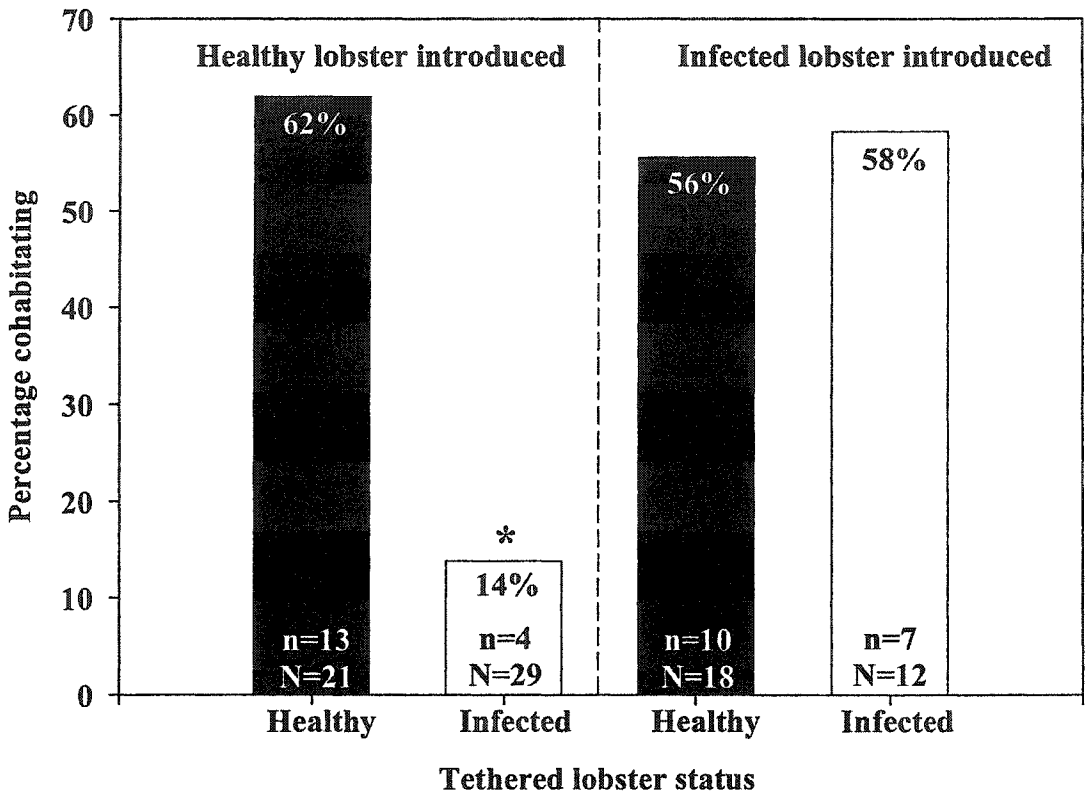

FIG. 18. Results of the laboratory "den choice" experiment wherein non-infected and infected juvenile lobsters were given a choice of sheltering alone or with another lobster (either infected or non-infected) that was tethered to one den (*, $P = 0.001$).

B. Disease transmission by ingestion and the impact of stress on transmission:

In the ingestion trial, 8% of the lobsters died within 45 days after having consumed diseased tissue. Subsequent histological examination of tissues from the lobsters surviving after 80 days indicated that another 33% were, in fact, infected with the HLV-PA virus. Thus, a total of 42% of the lobsters that were fed diseased tissue on four occasions during

the experiment eventually contracted the virus. All of the control lobsters were uninfected after 80 days. Evidence that the HLV-PA disease is more virulent among the earliest juvenile stages was also seen in this trial, wherein five of the six smallest lobsters contracted the disease. Two of those infections resulted in death within 80 days. Salinity stress did not have a significant effect on the transmissibility of the virus by ingestion (X^2 = 2.983, df = 3, P = 0.394).

C. Disease transmission by contact or waterborne means:

Histological examination of the lobsters that were alive after 80 days in the contact/waterborne transmission experiment indicated that 30% of those exposed to HLV-PA infected conspecifics became infected themselves. Only two of these lobsters (7%), both from the small (20 – 30 mm CL) size group, were sufficiently diseased that they could be diagnosed by visual means. The randomized block ANOVA used to evaluate differences in infection level among groups of lobsters of different size was significant ($F_{2,8}$ = 4.678, P = 0.026); 63% of the small lobsters (20 – 30 mm CL), 33% of the medium lobsters (30 – 40 mm CL) and 11% of large lobsters (40 – 50 mm CL) became infected with HLV-PA. The small lobsters had a significantly greater infection level than the large lobsters (Tukey's HSD). The medium lobsters did not have an infection level significantly different than either the small or the large lobsters. The block effect (i.e., tank effect) was not significant ($F_{8,15}$ = 1.525, P = 0.229). The size-specific pattern of infection in this transmission experiment again suggests that risk of infection decreases with increasing lobster size. Two control lobsters from the small-size group were histologically diagnosed with HLV-PA infections after 80 days. These individuals, obtained from the field, were probably infected at the initiation of the experiment since

all lobsters deemed healthy at that time were only assessed by visual means. It is quite probable that two of the 15 control lobsters would already be infected considering that my field surveys revealed that 16% of wild lobsters of this size (< 20 mm CL) were diseased.

Discussion

The HLV-PA virus altered the behavior of infected juvenile spiny lobsters, and perhaps more remarkably, the behavior of their healthy conspecifics. Healthy individuals avoided infected conspecifics, which presumably is an adaptation to reduce their risk of infection. The results of the contact/waterborne transmission trials lend credence to this by providing evidence that the virus was directly transmitted among individuals that were in close proximity. Whether viral transmission required physical contact or could be achieved through waterborne means, and if so over what distances, remains unknown. However, I speculate that the transmissibility of the virus need not be high because of the social nature of spiny lobsters.

Direct impacts of disease on social animals

Pathogens, especially those directly transmitted among individuals, are generally more prevalent in gregarious animals (Davies et al. 1991, see Cote and Poulin 1995 for review, Porteous and Pankhurst 1998, Poulin and Rate 2001), with the benefits of sociality (e.g., predator defense, locating resources, reproduction, etc.) outweighing the detriment of increased infection (Loehle 1995). Social animals have adapted to this increased risk of infection with an increased investment in immune function (Moller and

Erritzoe 1996, Moller et al. 2001, Brown and Brown 2002) and increased genetic variation (i.e., polymorphism) through mechanisms such as polyandry (Sherman et al. 1988, Baer and Schmid-Hempel 2001). If only a portion of the colony possesses a genetic resistance to the infection, then the possibility that a parasite or pathogen could spread sufficiently to jeopardize colony survival is diminished. Evidence even suggests that a social organism, the dampwood termite (*Zootermopsis angusticollis*), actually gains immuno-resistance from association with immunized nestmates (Traniello et al. 2002). Similarly, some soft corals (*Gorgonia ventalina*) develop an increased resistance to the pathogenic fungus *Aspergillus sydowii* following inoculation with the fungus (Dube et al. 2002). O'Donnell (1997) actually proposes that parasites may promote sociality in systems where reproductively detrimental parasites (i.e., those that castrate or reduce the fecundity of the host) are abundant, acquired outside of the nest, and are not directly transmittable between conspecifics. Under these circumstances, parasitized individuals are more likely to forgo reproduction and assume the role of a worker, thus promoting sociality.

Animals, whether social or not, have developed an extensive battery of behaviors (e.g., grooming, preening, bathing, maintenance of sanitary habitat, etc.) to avoid infection by pathogens (see Loehle 1995 for review). Considering the obvious advantages that detection and avoidance of diseased conspecifics would afford, there is surprisingly very little empirical evidence that this mode (i.e., avoidance of diseased conspecifics) of disease resistance has evolved in animal populations. Except for the evidence presented here, I know of only one other study wherein this behavior has been documented. Kiesecker et al. (1999) demonstrated that healthy bullfrog tadpoles (*Rana*

catesbeiana) avoid those infected with a parasitic yeast (*Candida humicola*). Furthermore, they state that their study is the first to document such behavior. Early epidemiological theory posits that directly transmitted diseases are selectively neutral because all members of the population are equally susceptible (Kermack and McKendrick 1927). However, many animals have evolved behaviors to reduce mortality from factors such as predation, so it is reasonable to assume that in certain circumstances they may have also developed behaviors to avoid another potential source of mortality, such as disease. Both predators and pathogens may drive selection for characteristics that reduce their effect, and both are capable of co-evolving in the face of these adaptations (i.e., Red Queen Hypothesis, Van Valen 1973). Animals have evolved behavioral as well as physical attributes to avoid predation such as flight responses, evasive maneuvers, cryptic behavior or coloration, body armor, limb autotomization, and sociality. Similarly, they have evolved behavioral and physical characteristics to avoid pathogens such as grooming, sanitation, impenetrable integument, cell-mediated and humoral immune responses, and physiological and behavioral fevers. Thus, evolving the ability to discriminate diseased conspecifics should be a characteristic particularly beneficial to animals wherein sociality is deemed essential to fitness (i.e., courting and mating activities, group defense strategies, location of important resources) (Loehle 1995).

Herein lies the paradox. The benefits derived from sociality have been purported to negate the selective advantage of being able to detect and avoid diseased conspecifics (Brown and Brown 1986, Loehle 1995). Increased risk of infection has been viewed as a mere cost of sociality (Loehle 1995).

Direct impacts of disease on spiny lobsters

Spiny lobsters become social after reaching ~15 mm CL (Andree 1981, Childress and Herrnkind 1994, 1996, Butler and Herrnkind 1997), when they seek out and often share with conspecifics any available, appropriately-sized crevice (Forcucci et al. 1994, Herrnkind et al. 1997*b*). Cohabitation is not obligatory because 45% of the juvenile lobsters that I observed in the natural hard-bottom environment of the Florida Keys were solitary. However, 13% of those solitary lobsters were visibly diseased and an unknown number had probably contracted the diseased but had not yet displayed visible signs of infection. This level of cohabitation is consistent with previous field estimates of the frequency of shelter co-occupancy by juvenile lobsters (Childress and Herrnkind 1996, 1997). The "guide effect" is one explanation that has been proposed to explain the somewhat contradictory observation that highly social juvenile *P. argus* are only found cohabitating in the field about half the time (Childress and Herrnkind 1997, 2001). That hypothesis suggests that juvenile lobsters do not benefit from aggregation in shelters through decreased predation (i.e., through dilution or cooperative defense), but use the odor of conspecifics to locate shelter more rapidly, thus reducing the time spent in the open searching for shelter. Evidence offered in support of this hypothesis is that the distribution of lobsters is rarely other than random and only correlated with shelter availability and conspecific density, not predator density. Although this explanation for the observed dispersal of juvenile lobsters is plausible, the presence of disease in a lobster population may also diminish the aggregation, perhaps sufficiently so to alter the spatial distribution of the population from a statistically clumped (underdispersed) to a random pattern. My colleagues and I intend to examine this issue further in a series of computer

simulations wherein population spatial patterns are investigated under varying degrees of disease prevalence, shelter availability, and shelter spatial distributions.

In Caribbean spiny lobsters, the same ability that promotes gregariousness - attraction to the chemical cues of conspecifics - (Zimmer-Faust et al. 1985, Zimmer-Faust and Spanier 1987, Childress and Herrnkind 1996, Ratchford and Eggleston 1998, 2000, Butler et al. 1999) may have permitted selection for avoidance of diseased conspecifics if they emit a detectably different compound when ill. The transmission studies showed that HLV-PA was transmitted among individuals in close proximity (especially among those lobsters < 20 mm CL), making avoidance a logical method for reducing infection. Furthermore, the fact that macroalgal-dwelling, cryptically-colored juveniles are asocial and later become social and lose this coloration (Andree 1981) may be as much related to their lack of disease resistance as to avoidance of predators. Both predation susceptibility (Smith and Herrnkind 1992, Childress and Herrnkind 1994) and infection susceptibility may have played a role in the evolution of this behavioral change, as it is hard to reconcile the commensurate ontogenetic change in camouflage coloration if avoidance of visual predators is not a significant factor.

The ability of healthy lobsters to detect and avoid infected conspecifics appears to be lost among infected lobsters, a situation similar to that observed by Kiesecker et al. (1999) among bullfrog tadpoles. This lack of response is likely due to an impaired ability to detect the cue that healthy individuals are using to avoid diseased conspecifics (Lefort and Blaustein 1995, Kiesecker et al. 1999). Spiny lobsters are highly sensitive to chemical cues, using them to both facilitate aggregation (Zimmer-Faust et al. 1985, Zimmer-Faust and Spanier 1987, Childress and Herrnkind 1996, Ratchford and Eggleston

1998, 2000, Butler et al. 1999) and in predator detection (Berger and Butler 2001). The actual cue utilized by juvenile lobsters to detect infection in conspecifics is unknown.

Indirect impacts of disease

Not all impacts of disease on populations are a direct result of disease-induced mortality. The indirect impacts of disease on population dynamics are often more subtle, and more difficult to measure. Increased susceptibility to predation is a common theme in studies of the alteration in behavior of animals infected with a pathogen. Pathogen-induced reductions in host fecundity or host sterilization are also reported (Sindermann and Farrin 1962, Sindermann 1965, Dobson 1988, Moore and Gotelli 1990), but this does not apply to HLV-PA infection which has only been observed in juvenile lobsters. Increased susceptibility to predation due to infection is often the result of increased physical or behavioral conspicuousness, depressed anti-predatory behavior, or morbidity and is hypothesized to benefit the pathogen by enhancing transmission, especially if the pathogen is a parasite that requires an intermediate host for completion of the life cycle (see Holmes and Bethel 1972, Dobson 1988 and Poulin 1995 for reviews). Numerous additional examples exist demonstrating increased predation on animals infected by macroparasites. For example, Moore (1983) found that isopods (*Armadillidium vulgare*) infected with the acanthocephalan (thorny-headed worm) parasite (*Plagiorhynchus cylindraceus*) behave differently than non-infected isopods, resulting in their increased consumption by the definitive host, the starling (*Sturnus vulgaris*). A recent study on the rough periwinkle (*Littorina saxatilis*) infected with the trematode, *Microphallus piriformes*, found that infected periwinkles moved higher on intertidal rocks during tidal

cycles than non-infected conspecifics, presumably resulting in enhanced predation rates by the ultimate host, the herring gull, *Larus argentatus* (McCarthy et al. 2000). This behavior is similar to that observed in the estuarine gastropod, *Ilyanassa obsoleta*, when it is parasitized by larvae of the trematode, *Gynaecotyla adunca* (Curtis 1987, 1990). Snails infected with *Gynaecotyla adunca* are found farther up in the intertidal zone than non-infected conspecifics, often becoming stranded there during low tide, which aids in the transfer of the parasite to an intermediate semi-terrestrial crustacean host (e.g., the beach hopper amphipod, *Talorchestia longicornis* and the fiddler crab, *Uca pugilator*). A variety of shore birds can act as the definitive host by ingesting the crustacean. The preponderance of altered host behaviors in macroparasite-infected hosts is hypothesized to result from the complex host and predator-prey interactions in which they have often evolved (Moore and Freehling 2002). I do not yet know whether the HLV-PA virus infects other hosts, but I suspect that predators of juvenile lobsters, (e.g., bonnethead and nurse sharks, stingrays, octopus, toadfish and grouper; Smith and Herrnkind 1992) are unlikely to contract the virus due to the host specificity common to many invertebrate viruses (see Adams 1991 for review).

Pathogen-altered host behaviors that result in enhanced predation on the host by intermediate hosts are intriguing and often spectacular. However, changes in host behavior that increase host susceptibility to predation in the absence of intermediate hosts are less frequently documented, and are often a by-product of a non-specific response to infection or stress (Horton and Moore 1993, Lefcort and Blaustein 1995, Poulin 1995). The pathogen defense response, termed the acute phase response, includes symptoms such as fever (physiological or behavioral), lethargy and malaise (Lefcort and Eiger

1993). Though hypothesized to enable an organism to fight an infection by inhibiting the pathogen (Hart 1988, 1990, Lefcort and Eiger 1993), the acute phase response behavior may increase mortality by increasing the susceptibility of the host to predation. Lefcort and Eiger (1993) used alcohol-killed bacteria (*Aeromonas hydrophila*) to elicit normal pathogen defense responses in bullfrog tadpoles (*Rana catesbeiana*) and found these responses to result in enhanced predation by the roughskin newt (*Taricha granulosa*). Similarly, Lefcort and Blaustein (1995) used the directly transmitted yeast parasite (*Candida humicola*) to elicit behavioral alterations in red-legged frog tadpoles (*Rana aurora*) that also resulted in increased predation by *T. granulosa*. In these examples, the pathogen does not benefit from the consumption of the host. This is especially common for bacterial and viral infections that are often host-specific.

Nonetheless, the transmission of several diseases that are directly transferred among congeneric hosts is increased by pathogen alteration of host behavior. The rabies virus, induces behavioral shifts and neurological pathologies in its mammalian host, which expedites its transfer through unprovoked bites (MacDonald 1980). Goulson (1997) described a less obvious case where larval moths, *Mamestra brassicae*, infected with the *Mamestra brassicae* nuclear polyhedrosis virus dispersed to a greater degree than non-infected larvae. Moreover, their position upon death, at the top of trees and at the tips of the leaves, was thought to enhance transmission of the virus, as rainfall washing over the lyzed larvae dispersed the virus over the vegetation below, which in turn was ingested by other larvae.

Indirect impacts of disease on spiny lobsters

A. Predation:

Increased mortality due to direct predation on infected individuals may increase the probability of predation on lobsters in the advanced stages of infection, when they are lethargic and moribund, and presumably less able to evade predators. The notable lethargy associated with advanced disease is probably due to the depletion of energetic reserves (i.e., glycogen in reserve inclusions) and ischemia (see Chapter II) combined with depressed nutritional condition. The latter may be due in part to a lack of feeding noted as lobsters become visibly infected. Increased predation on a behaviorally-compromised host (e.g., HLV-PA infected lobster) by predators that are immune to infection could actually reduce transmission of the virus if sufficient numbers of infected hosts are culled from the population. However, the opposite may be true if the pathogen remains viable in the feces produced by the predator and is spread in this manner to new locations.

As the HLV-PA infection progresses in an individual, it may also preclude a lobster from benefiting from the reduction in predation risk associated with gregariousness. The ontogenetic shift for juveniles at ~15 mm CL from an asocial macroalgal-dwelling phase (Andree 1981) to a gregarious post-algal crevice-dwelling existence is hypothesized to reduce their susceptibility to predation through group defense (i.e., dilution, increased vigilance and cooperative defense) (Eggleston and Lipcius 1992, Mintz et al. 1994, Butler et al. 1997, 1999, but see Childress and Herrnkind 1994, 1996, 1997). To date, I have only observed lethargy in isolated lobsters with late-

stage infections, and do not know if lobsters with earlier stages of infection are impeded from participation in group defense. Once infection reaches the stage (yet undetermined) when healthy lobsters avoid them, their solitary existence alone may expose them to a greater risk of predation than is the case for cohabitating lobsters (Butler et al. 1999).

B. Shelter availability:

A demographic bottleneck is a phase in the life history of an organism that limits the future size of the population (Caddy 1986, Caddy and Stamatopolous 1990). This concept was first applied empirically to lobsters in a study of habitat limitation to recruitment, though other organisms have been shown to be shelter limited (e.g., stomatopods; Steger 1987). Wahle and Steneck (1991) found juvenile American lobsters (*Homarus americanus*), 5 – 40 mm CL, to be limited by the availability of their refuge habitat, cobble substrate. Similarly, post-algal stage juvenile *P. argus* appear to be limited in some regions by available crevice shelters (Butler and Herrnkind 1997). Depending on the age or life history stage where the limitation occurs, it can affect not only survival but growth and fecundity as well. For example, adult stone crabs (*Menippe mercenaria*) depend on appropriately sized shelters for both molting and egg production (Beck 1995, 1997). In areas augmented with shelters, crabs molt more frequently and females produce eggs faster than in areas without sufficient shelter. Shelter limitation is not a phenomenon exclusive to crustaceans, having been demonstrated in vertebrates as well. Populations of reef fish (Hixon and Beets 1993, Schmitt and Holbrook 2000) and crevice-dwelling birds (Brawn and Balda 1988), for example, can also be limited by available refuge.

Juvenile lobsters are highly vulnerable to predators irrespective of shelter availability (Smith and Herrnkind 1992, Herrnkind et al. 1997*b*). Moreover, it is typically the size or stage of the organism with the highest risk of predation that is shelter limited (Wahle and Steneck 1991, Butler and Herrnkind 1997, Beck 1995). As noted above, crevice shelters used by juvenile lobsters to avoid predation are known to be limited in some areas of Caribbean and their scarcity could create a demographic bottleneck (Butler and Herrnkind 1997). If an infected lobster takes up residence in a shelter and other lobsters that normally would cohabitat with that individual instead avoid it, then an effect of the virus could be to further limit shelter availability in these areas. Since infected juveniles were normally alone (mean = 91%), high prevalence combined with low shelter availability could act synergistically to exacerbate the effect.

The potential benefit accrued by non-infected lobsters capable of detecting and avoiding virus-infected conspecifics is obvious, because the pathogen is directly transferred among individuals. Yet, further investigation of the dynamics of transmission is necessary to fully understand the risk of infection under different environmental circumstances and the actual effectiveness of the avoidance behavior seen in healthy lobsters in reducing infection risk.

Transmission

A. Inoculation and ingestion transmission:

HLV-PA was successfully transmitted via serial inoculation between infected and healthy lobsters, with 95% of lobsters becoming infected after 80 days as determined by histological examination. The oral ingestion trial was also successful, after 80 days 42%

of lobsters were confirmed through histological diagnosis to have become infected with HLV-PA, or previously succumbed to the infection and died. Although the focus of this experiment was to test viral transmissibility via ingestion, I could not rule out the possibility that infections were derived from virions released from the infected food and suspended in the water column.

B. *Contact or waterborne transmission:*

Many viruses in both fresh and marine systems remain viable and infectious in the water column. For example, the haematopoietic necrosis virus is transmitted to spawning sockeye salmon (*Oncorhynchus nerka*) from the water column (Mulcahy et al. 1983), as is the penaeid rod-shaped DNA virus (PRDV) infecting penaeid shrimp (Wu et al. 2001). The freshwater crayfish, *Astacus astacus*, experimentally infected with the infectious pancreatic necrosis virus (IPNV) continually shed viral particles into the water column, infecting the eggs and fry of the rainbow trout (*Salmo gairdneri*) (Halder and Ahne 1988). Deciphering the exact mode of transmission in the contact/waterborne transmission trial that I conducted on juvenile *P. argus* is problematic. Transmission could have been through the water, through contact, or through ingestion of infected fecal matter. An isolated waterborne transmission trial in which healthy lobsters are exposed to the effluent from tanks in which diseased lobsters are held is planned for 2003.

Prevalence and distribution

The prevalence of the HLV-PA virus in populations of the juvenile spiny lobster within nursery habitat sites in the middle and lower Florida Keys remained consistent from one survey to the next, varying by only 1.1% between summer 2000 to summer

2001. Prevalence data represent only those individuals with late-stage, visually diagnosable infections and are, thus, underestimates of the actual number of infected lobsters at each site. The eventual development of an immunological- or genetic-based assay for determining infection will solve this problem, and we are currently working on developing such an assay. Nevertheless, based on comparisons between visually estimated disease prevalence versus histologically confirmed infections in my laboratory experiments, I suspect that actual infections may be 25% higher in the field than reports based on visual estimation. In other words, the actual prevalence of the disease in the Florida Keys is probably in the range of 8-10%.

The prevalence of HLV-PA among juvenile lobsters in south Florida warrants concern. Shields (1994) used 10% as an arbitrary level for defining epizootics in parasitic dinoflagellates in crustaceans since the level at which a mortality event is considered a mass mortality or epizootic is often difficult to determine (Sindermann 1990). The HLV-PA virus infects approximately 16-20% of the lobsters below 20 mm CL, so this should be considered an epizootic with potentially serious implications for future adult populations. Since an estimated 90% of all adult lobsters are harvested from the Keys during each season (Hunt 2000), the link between the HLV-PA prevalence estimates and the present state of the fishery is cause for alarm. In fact, the 2000-2001 (2.5×10^6 kg) and 2001-2002 (1.4×10^6 kg) harvest levels for *P. argus* in Florida are two of the lowest levels in 30 years (Hunt, *personal communication*). I do not know to what extent the virus has contributed to this down-turn in the fishery, but the coincidence with the disease is ominous and no other explanations for the decline appear sufficient. There have been no major changes in nursery habitat suitability of which I am aware, unlike a

decade ago when the region experienced a major die-off of sponges that provide shelter to lobster (Butler et al. 1995, Herrnkind et al. 1997*b*). Similarly, the supply of postlarvae to the region did not decline precipitously in the years leading up to the drop in fishery landings, nor has fishery effort increased. In fact, fishery effort has decreased by 46% in the past decade due to an effort reduction program mandated by fishery managers (Hunt 2000).

Hypotheses for HLV-PA limitation to juvenile *P. argus*

The predilection of the virus for juvenile lobsters may have several explanations. In a survey performed by the Florida Fish and Wildlife Conservation Commission in July, 2001, and October, 2001, 860 and 667 adult lobsters were surveyed, respectively, from offshore reefs throughout the Florida Keys. Of these 1527 lobsters, only four individuals (< 1%) presented visual signs of HLV-PA infection during the July survey and none were recovered during the October survey. However, nearly all of the infected animals were sub-adults (70 - 74mm CL) that may have recently migrated to the reef from the nursery, thus providing further evidence that HLV-PA is limited to juvenile lobsters. This condition is not unique to HLV-PA though, as numerous pathogens infect a specific age or life history stage of an organism or, in other cases, the susceptibility of the organism changes with age or stage. The parasitic dinoflagellate *Hematodinium* sp. infects small (< 30 mm CL) blue crabs (*Callinectes sapidus*) significantly more than larger crabs (Messick and Shields 2000). Small false king crabs (*Paralomis granulosa*) were also found to have a higher prevalence of the parasitic bopyrid isopod, *Pseudione tuberculata*, than large crabs (Roccatagliata and Lovrich 1999). Conversely, resistance to

the pathogenic fungus, *Aspergillus sydowii*, in the soft coral *Gorgonia ventalina* decreases with age (Dube et al. 2002). Juvenile oyster disease (JOD) appears to be linked to small oyster size, irrespective of age, though the exact etiological agent has not been identified (Ford and Borrero 2001). This phenomenon is not limited to the animal kingdom; plants also show differential susceptibility with age (Garcia-Ruiz and Murphy 2001).

A. Physiological hypothesis:

Due to the proliferation of studies showing age- or size-based resistance or susceptibility, I speculate that HLV-PA infection of *P. argus* involves a decrease in lobster susceptibility to infection with age. Smaller lobsters grow faster than larger lobsters, and must molt frequently to do so. The molting process is an energetically and physiologically taxing process that small crustaceans of questionable health may not survive (i.e., molt-death syndrome; Bowser and Rosemark 1981). Frequent molting may increase the susceptibility of smaller lobsters to HLV-PA due to the nearly continual physiological stress of molting, or ease with which the virus may penetrate the exoskeletal defenses of the host before the carapace of the lobster has hardened. Messick and Shields (2000) proposed this same mechanism to explain the higher prevalence of the parasitic dinoflagellate, *Hematodinium* sp., in small blue crabs (*Callinectes sapidus*).

B. Habitat specificity hypothesis:

Another possible explanation for the difference in the prevalence of HLV-PA in juvenile versus adult lobsters is that different life stages occur in different habitats. The majority of the juvenile population resides in shallow, hard-bottom habitat in western Florida Bay and the shallow waters surrounding the Florida Keys islands. In contrast,

adult lobsters dwell primarily on coral reefs that lie 7 – 10 km south of the Florida Keys archipelago. This ontogenetic shift in lobster habitat use could explain the lack of HLV-PA among adults if a viral reservoir exists in other species not found on the reef, or if the virus itself is sensitive to differences in environmental characteristics (e.g., depth, water quality, temperature, salinity, sedimentary characteristics) that differ between these regions (Holmquist 1989b, Boyer et al. 1997, Bosence 1989). Many of these characteristics are subject to rapid change in the nursery region primarily due to its bathymetry (i.e., mean depth 1-2 m). Should environmental stress emerge as a factor in the presence or prevalence of HLV-PA, these conditions would be most severe in the nursery regions.

Salinity, especially when combined with extreme temperatures, has a dramatic impact on the survival of postlarval and early benthic stage lobsters (Field and Butler 1994, Butler et al. *unpublished data*), but does not affect the survival of larger (> 30 mm CL) juvenile lobsters (Butler et al. *unpublished data*). Large juvenile lobsters of this size appear much more capable of adapting to variations in salinity (i.e., osmoconforming) or move out of these areas when salinities approach the extremes (Butler, *unpublished data*). This ability to adapt to changing salinities may explain the lack of significance that I observed in viral transmission among lobsters exposed to various salinity treatments in the ingestion trial. Moreover, though portions of central and eastern Florida Bay experience extremes in salinity (Boyer et al. 1997) that can range from 15 – 50 psu annually, the majority of the spiny lobster nursery habitat lies to the south and west of this area.

Conclusions

Whatever the cause, HLV-PA is at disturbingly high levels among the smallest lobsters, it can be transmitted by ingestion of diseased tissue or close contact with infected individuals, and it is highly pathogenic once acquired. The disease may also indirectly impact healthy juvenile lobsters through its effects on the dynamics of shelter use and co-occupancy. The avoidance of diseased conspecifics by healthy lobsters is striking and likely has the beneficial effect of limiting transmission of this lethal virus, thus tempering its prevalence. The dynamics of this disease are indeed intriguing and stand to alter our perceptions of the role of disease in structuring populations of social animals – yet its implications for fisheries are disturbing. Spiny lobsters support one of the most valuable fisheries in Florida and the Caribbean. HLV-PA infects a substantial fraction of the juvenile lobsters in Florida, yet its occurrence and potential impact on lobster populations elsewhere in the Caribbean are unknown.

CHAPTER IV

THE IMPACT OF ARTIFICIALLY ENHANCED DENSITY ON JUVENILE SPINY LOBSTER NUTRITIONAL CONDITION, SHORT-TERM RESIDENCY AND DISEASE

Introduction

Research on the artificial enhancement of habitat for marine fisheries includes studies on habitat design (Briones-Fourzán et al. 2000, Cruz and Phillips 2000, Sosa-Cordero et al. 1998 Nedimyer et al. 2001, Seaman 2000, Losada-Torteson and Posada 2001, Sherman et al. 2001*a*), the proper size and number of habitats (Seaman 2000, Sherman et al. 2001*b*), the location of habitats (Sosa-Cordero et al. 1998) and the success of a given artificial habitat in preserving population persistence or improving exploitation efficiency (Cruz et al. 1986, Tangley 1987, Coen and Luckenbach 2000, Seaman 2000, Losada-Torteson and Posada 2001, Nedimyer 2001). At the core of many of these studies lies the "attraction vs. production" controversy. That is, the extent to which artificial enhancement of habitat increases organism abundance (or production) via reduced mortality or enhanced growth, or whether it simply concentrates individuals from surrounding areas potentially placing them at increased risk of fishing mortality (Bohnsack 1989, Lindberg 1997, Bortone 1998, Johnson 2001). In evaluating artificial structures, most investigators have studied the efficacy of artificial enhancement by measuring population abundance of the target species or by comparison of species

richness and diversity of communities recruiting to artificial structures in relation to unmanipulated natural regions, (e.g., Bohnsack et al. 1994, Lozano-Alvarez et al. 1994, Stanley and Wilson 2000, Briones-Fourzan and Lozano-Alvarez 2001). Few studies have examined the impact of artificially enhanced local population density on population dynamics such as movement, growth, nutritional condition, or susceptibility to disease.

Lobsters, both spiny and clawed, rely on crevice shelters for refuge (Wahle and Steneck 1991, Herrnkind and Butler 1986, Smith and Herrnkind 1992, Herrnkind et al. 1994, Polovina et al. 1995, Butler and Herrnkind 1997) and this dependence has been both implicitly (i.e., through loss of natural habitat) (Herrnkind et al. 1997*b*) and explicitly (i.e., through addition of artificial habitat) (Butler and Herrnkind 1997, Herrnkind et al. 1997*a*) demonstrated to limit juvenile spiny lobster populations. The availability of appropriately-sized crevice shelters (Eggleston et al. 1990, Eggleston and Lipcius 1992, Mintz et al. 1994) has even been suggested to be a possible "bottleneck" to future adult population size (Wahle and Steneck 1991, Butler and Herrnkind 1997, Herrnkind et al. 1997*a*). This dependence of juvenile spiny lobsters on the presence of adequate crevices for sheltering permitted me to address the effects of density on population dynamics through the addition of artificial habitat in field experiments.

Several studies have demonstrated both positive (Jorstadt et al. 2001) and negative (Booth and Kittaka 2000, James et al. 2001) density-dependent growth and survival in crustaceans held in artificial laboratory conditions, but there is little evidence for density-dependent impacts among mobile macroinvertebrates in the field. This may be due, in part, to difficulties in manipulating wild populations or in accurately assessing nutritional condition or growth. Wahle et al. (2001) found that the growth of American

clawed lobster (*Homarus americanus)* was depressed at high stocking densities in field enclosures, although the mechanism producing the result (including possible caging artifacts) was unknown.

For crustaceans where natural density-dependent population dynamics are known, most evidence points to increased emigration at high population density. The Dungeness crab, *Cancer magister*, emigrated at significantly higher rates when at high experimental densities (Iribarne et al. 1994). Similarly, a significant relationship between density and emigration into the water column was found in the infaunal amphipod, *Rheoxynius abronius* (Ambrose 1986). An intriguing study on density-dependence in the Western Australian rock lobster (*Panulirus cygnus)* attempted to experimentally decrease lobster density on several patch reefs in western Australia (Ford et al. 1988). Although they observed decreased mortality on low-density reefs, their estimates of mortality on unmanipulated high-density reefs are in doubt due to their inability to differentiate between mortality and emigration to other non-experimental reefs. However, their recapture rates on the low-density sites were very high, suggesting a lack of emigration at low density. This combined with the possibility that lobsters emigrated from the high-density reefs suggests that otherwise social spiny lobsters may emigrate when at high density, possibly to avoid intraspecific competition and the resulting nutritional deficits.

Increased population density can also facilitate the transmission of pathogenic diseases in macroinvertebrate populations. Goyer et al. (2001) found a significant relationship between population density of larval leafrollers, *Archips argyrospila*, and viral infections. At low to moderate density, the sheltering leaf rolls created by the larvae protect them from infection, but this benefit is lost at high population densities. In the

extreme, such a relationship can develop into a system of positive and negative feedbacks potentially resulting in population regulation. For example, population cycling of the stream-dwelling caddisfly *Brachycentrus americanus* appears to be regulated through density-dependent infection by a microsporidian parasite (Kohler and Holland 2001). However, disease is not always the most prevalent in the most dense animal populations. When western tent caterpillar colonies were experimentally challenged with infected individuals, no relationship was seen between the proportion of new infections per infected caterpillar introduced and colony member density (Beisner and Myers 1999). There is even evidence that in some cases crowding can enhance the resistance of an organism to infection with a pathogen. Wilson et al. (2002) found that desert locust (*Schistocerca gregaria)* are more resistant to infection by entomopathogenic fungus (*Metarhizium anisopliae* var. *acridum)* when reared in dense aggregations rather than solitarily. They propose that individuals capable of using population density as a cue to regulate the allocation of resources for disease resistance are favored through natural selection. This condition was first documented in a study of the phenotypically plastic lepidopteran (*Spodoptera exempta)* infected by a baculovirus and was termed "density-dependent prophylaxis" (Wilson and Reeson 1998, Reeson et al. 1998).

In general, density-dependent relationships between hosts and their pathogen are rare in nature and highly dependent upon factors such as the mode of disease transmission, physiological or behavioral changes in host characteristics that are induced by infection, and the generation time of the host or pathogen. However, if present, the role of density in the dynamics of a host-pathogen relationship can be substantial, especially in gregarious taxa (Duffy 1983, Brown and Brown 1986, Moller et al. 2001,

see Cote and Poulin for review). Spiny lobsters, for example, exhibit an ontogenetic shift in social behavior so that late-stage juveniles and adults aggregate with conspecifics in shelters by day (Herrnkind et al. 1975, Childress and Herrnkind 1994, 1996, Ratchford and Eggleston 1998, Butler et al. 1999). The discovery of a pathogenic virus infecting natural populations of juvenile spiny lobsters raises the possibility that density may play a role in the transmission of disease within this social species.

HLV-PA is a recently discovered pathogenic blood-borne herpes-like virus that infects juvenile lobsters at high rates of prevalence in the Florida Keys, USA (Chapter II). On average, about 10% of the juvenile lobsters were infected with the virus at 14 sites sampled over two years in the Florida Keys. The virus is lethal and gross symptoms of HLV-PA in advanced cases are lethargy, morbidity, cessation of molting and grooming, depressed nutritional condition (see Chapter II and III), loss of hemolymph clotting ability, and milky or chalky-colored hemolymph. The virus has been experimentally transmitted to healthy lobsters by injection with infected hemolymph, oral ingestion of disease tissue, and by direct contact with diseased individuals (Chapter III). Natural densities of juvenile *P. argus* in south Florida can exceed 700 individuals per hectare (Forcucci et al. 1994) and their social nature results in small-scale aggregations of lobsters that co-occupy crevice shelters (Childress and Herrnkind 1994, 1996). These are the kinds of conditions that could promote epizootic levels of infection if there is a positive relationship between density and HLV-PA viral transmission.

The dependence of juvenile spiny lobsters on the presence of adequate crevices for sheltering and the availability of artificial structures designed to mimic the shelter characteristics (e.g., size of structure and crevices, sheltering capacity) and spatial

distribution of natural crevices afforded me the opportunity to experimentally compare the effects of artificially enhanced and naturally varying differences in population density on population dynamics (i.e., nutritional condition, short-term residency and disease prevalence) and disease transmission of juvenile Caribbean spiny lobster. Thus, the objectives of this study were to: (i) determine if either natural or artificially enhanced density has any affect on the nutritional condition or short-term residency of juvenile lobsters, (ii) describe the relationship between density and the prevalence of HLV-PA infection in juvenile lobsters.

Methods

Laboratory procedures

The refractive index of raw hemolymph was used as an indication of serum protein level and thus the general nutritional condition of lobsters collected in the field at sites of varying population density. The technique was described by Musgrove (2001) for *Jasus edwardsii.* It utilizes the refractive index of raw hemolymph as an indication of serum protein level that can then be compared to a standard curve created with bovine albumin. Stewart et al. (1967) in a study of the American lobster, *Homarus americanus*, showed that blood serum protein is a reliable indicator of physiological condition. A study performed nearly simultaneous to mine, also tested the use of raw hemolymph refractive index as an index of nutritional condition in juvenile rock lobsters, *Jasus edwardsii*, in New Zealand (Oliver and MacDairmid 2001). They found that hemolymph refractive index responded to changes in food supply, and further, that blood refractive index reflected blood protein concentration and, thus, condition. The major caveat to the

use of hemolymph refractive index as a measure of lobster condition is its variability with molt stage (*Homarus americanus*: Barlow and Ridgeway 1969, Stewart and Li 1969, *Callinectes sapidus*: Lynch and Webb 1973, *Panulirus longipes*: Dall 1974, *Homarus gammarus*: Hepper 1977, *Jasus edwardsii*: Oliver and MacDairmid 2001). The physiology of molting dictates that during the period leading to ecdysis (premolt) and for a brief period following ecdysis (postmolt), the hemolymph volume is altered (concentrated during premolt and diluted just prior and briefly after) which results in a corresponding change in the concentration of protein in the hemolymph (Smith and Dall 1982, Depledge and Bjerregaard 1989). Therefore, it is essential that molt stage be assessed when measuring hemolymph refractive index as a proxy for nutritional condition. Prior to employing the hemolymph refractive index technique in field experiments, I conducted a laboratory experiment to determine whether the technique was applicable to *P. argus*.

In June of 1999, I captured 40 lobsters (35 - 50 mm carapace length; CL) from various locations in the Florida Keys (Florida, USA) and held them individually in floating baskets in a flow-through seawater system at ambient temperature (25 - 28°C) and photoperiod (approximately 14hr light; 10hr dark) for six weeks. Lobsters were initially fed a pre-weighed diet of shrimp and squid *ad libitum*. Any food remaining after 4 hrs was re-weighed to determine their mean maximum daily consumption. For the remainder of the study, 20 lobsters were fed a diet at 25% of this empirically determined maximum and twenty were fed at 100% of the maximum. Hemolymph refractive index was measured for each lobster at the initiation and termination of the experiment. To do so, I used a 25-gauge tuberculin syringe to draw 0.1 to 0.2 ml of hemolymph from the

pericardial sinus of the lobster and delivered this to a Leica hand-held industrial refractometer, read to within 0.5 units. The refractive index value was then compared to a standard curve developed using bovine albumin to determine the serum protein level. The standard curve was created by the serial dilution of a stock concentrated (30% protein) bovine albumin solution. The dilutions ranged from 2.0 to 18.0 mg/ml protein and fully overlapped the range of raw hemolymph refractive index values observed for *P. argus*.

Field procedures

The effect of population density on juvenile lobster population dynamics was studied at twelve hard-bottom sites (625 m^2 in area; depth 1 - 3 m) up to 7 km from shore north of the Middle Keys, Florida (Fig.19). Tropical hard-bottom is the preferred habitat of juvenile spiny lobsters in the Florida Keys and is characterized by a thin veneer of sediment covering calcareous rock. Bushy, red macroalgaes (*Laurencia sp.*) are the dominant producers (Behringer and Butler, *unpublished manuscript*), whereas sponges, octocorals, and scleractinian corals are the most conspicuous sessile fauna; in conjunction with crevices in the substrate itself, these structures provide the majority of shelter for lobsters (Butler et al. 1995, Herrnkind et al. 1997*a*). Sites were designated as being one of three types based on the abundance and types of crevice-bearing structures that were present: (a) unmanipulated "natural" (NAT) sites, (b) "low density manipulated" (LDM) sites into which 12 - 18 artificial structures were added, and (c) "high density manipulated" (HDM) sites to which 25 - 50 artificial shelters were added. Each artificial structure that I placed on the manipulated sites consisted of a double-stacked concrete

partition block (40 cm x 20 cm x 10 cm) with three oval holes (10 cm x 4 cm) that approximated the overall size and crevice dimensions of natural shelters. Previous studies (Butler and Herrnkind 1997) have shown that these structures attract similar sizes and numbers of juvenile lobsters as natural shelters. For ease of sampling, the artificial structures were arranged in a rectangular matrix on each site with no structure situated closer than 2 m to another.

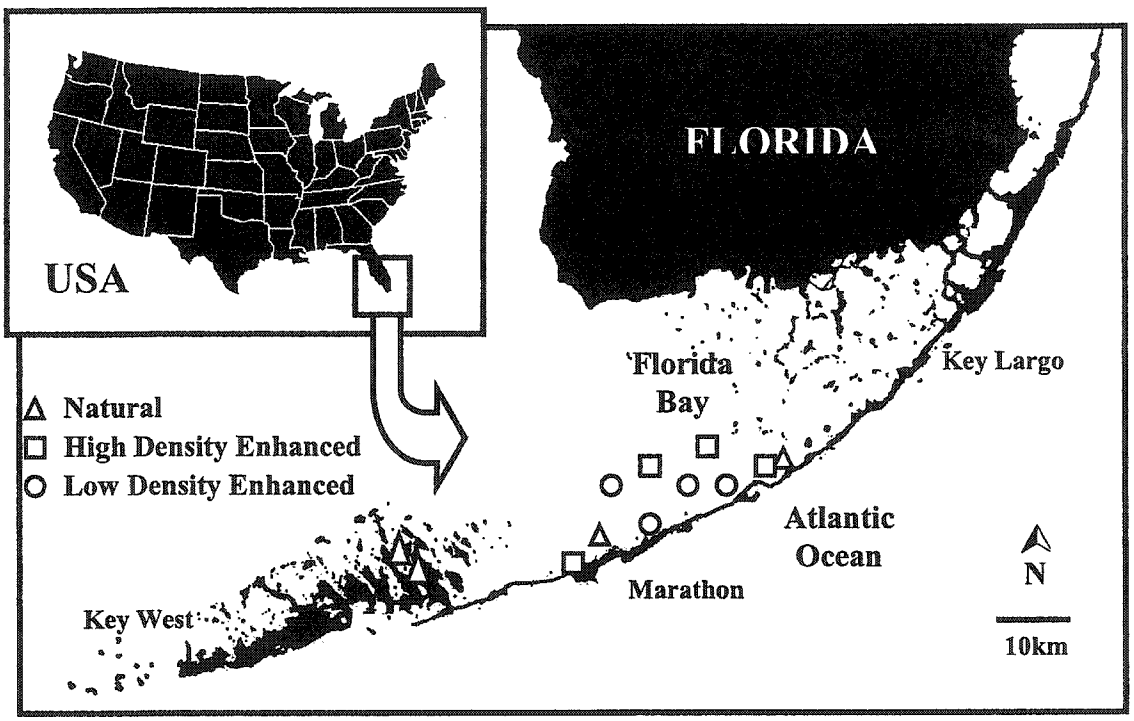

Fig. 19. Study field site locations. Triangles represent field site locations north of the Middle Keys, Florida (USA). Sites were each 625 m^2 in area, approximately 1 to 7 km from shore, and ranged in depth from 1 to 3 m.

Mark-recapture surveys were performed on each site twice per year during the two most distinct seasons found in the subtropics: summer and winter. This seasonal sampling was conducted to account for possible seasonal differences in lobster density and condition as a result of environmental changes (e.g., temperature). Temperature has

a large affect on the survival and molt cycle of larval and post-larval lobsters (Field and Butler 1993, Matsuda and Yamakawa 1997, Moss et al. 2001) and thus could affect nutritional condition in juveniles.

Sites were surveyed by one diver who surveyed the entire 625 m^2 site and recorded all the necessary data underwater to minimize disturbance to the lobsters. All the lobsters encountered were captured, then marked with a unique banded antenna tag. For each individual, I recorded the following: sex, carapace length, health status (diseased or healthy as determined by visual inspection), type of shelter in which they were found, and the number of conspecific cohabitants with which they were found. Lobsters were then immediately returned to their original den. After the initial survey and marking episode, each site was re-surveyed five days later. The five-day period allowed for short-term movement of animals to and from the study site. The proportion of marked lobsters remaining on the site therefore provides an estimate of short-term residency. During the second census, the same measurements were taken, but all animals were collected and brought back to the laboratory, where I recorded their CL, molt stage (as described in Lyle and MacDonald 1983), injuries, wet weight, hemolymph refractive index, and the visual presence of hemolymph infection. Animals were later returned to the sites from which they were collected.

To account for differences in relative predation between sites, 10 randomly chosen juvenile lobsters were tagged, measured and tethered at each site. Lobsters were tethered by attaching a fishing swivel to the tail with a small cable tie. The swivel was then attached to a concrete brick via a 20 cm piece of 4.6 kg test monofilament fishing line, thus restricting the movement of tethered lobsters placed on the seafloor. Tethered

lobsters were then distributed haphazardly at least 2 m apart around each site and left for 2 days, after which time the bricks were retrieved and the number of surviving lobsters noted. This method yields an estimate of relative predation pressure (Herrnkind and Butler 1986, Eggleston et al. 1990, 1992, Pile et al. 1996) among sites. For my purposes, tethering information (i.e., proportion of lobsters killed per site) was compared among sites to account for potential losses in the local lobster population that were due to predation, as compared to those that may have emigrated from the site.

On each site I also characterized the general structure of the natural habitat to account for the potential among-site effects of shelter abundance and macroalgal bottom coverage on lobster density, movement, and disease. Natural habitat was characterized on each site with four randomly placed 2 m x 25 m belt transects within the site. Transect data was gathered by one diver swimming along a 25 m measuring tape with a 2 m pole held at the midpoint and perpendicular to the tape. Any structure greater than 20 cm was recorded. Structures included anything capable of sheltering a juvenile lobster (e.g., sponges, corals, octocorals, holes, etc.). The mean number of structures per transect was used to characterize the natural habitat of the site.

The same 25 m measuring tape was used by the diver to measure the macroalgal (*Laurencia* spp.) benthic coverage. The diver would swim along the length of the transect and record the lengths over which the tape was laying on *Laurencia* spp. This was also performed four times and the average cover per transect used to characterize the bottom coverage on the site.

Statistical analyses

A. Dietary intake influence on nutritional condition:

A 2 x 2 model-I repeated-measures ANCOVA was performed on data from the laboratory nutritional condition experiment to determine whether food treatment (25% and 100% daily ration) or experimental date (repeated measure: initiation and termination dates) significantly influenced hemolymph refractive index values once molt stage (covariate) was included in the analysis. The ANCOVA assumption of homogeneity of slopes was tested for the molt stage covariate. The molt stage covariate was not significant, presumably because there was little variation among individuals in molt stage when the refractive index measurements were taken. I therefore omitted the covariate from the analysis and analyzed the data instead as a 2 x 2 model-I repeated-measures ANOVA. A Shapiro-Wilk test and a Levene's test were used to test the normality and homogeneity of variances assumptions, respectively. After a square root transformation, the hemolymph refractive index and weight/CL ratio data met both assumptions of the ANOVA test.

B. Impact of artificial enhancement on juvenile lobster population dynamics:

A 3 x 4 model-I repeated-measures ANCOVA was used to determine whether the shelter manipulation treatments established in the field study truly resulted in different densities of lobsters as intended. The first factor in this analysis was treatment (high density enhanced, low density enhanced, and natural) and the second factor (the repeated-measures effect) was sampling date. The density of natural structures and percentage of macroalgal cover were included as covariates in the analysis. A Shapiro-Wilk test and a Levene's test were used on the data to test normality and homogeneity of variances

assumptions. The raw lobster density data were both normally distributed and the variances were homogeneous among treatments. The slopes of the covariates did not violate the ANCOVA assumption of homogeneity of slopes. However, the covariates of habitat structure and percentage macroalgal cover did not explain a significant amount of the variation in juvenile lobster density and were therefore removed from the analysis and the data re-analyzed as a 3 x 4 model-I repeated-measures ANOVA. A post hoc least significant difference (LSD) multiple comparison test was used following the ANOVA to determine which treatment means differed.

The same analysis was also performed on the mean hemolymph refractive index for lobsters on each site and the proportion of lobsters recaptured to determine if the nutritional condition or short-term residency of lobsters differed among experimental shelter treatments. Covariates of habitat structure and macroalgal cover were tested for the ANCOVA assumption of homogeneity of slopes, but since they did not explain a significant amount of the variability, they were removed from the analysis of hemolymph refractive index. The data were subsequently reanalyzed as a 3 x 4 model-I repeated-measures ANOVA. The natural habitat availability covariate was significant, although the percentage macroalgal cover was not, in the analysis of the short-term residency data, therefore only the latter was removed from the analysis. A Shapiro-Wilk test of normality and Levene's test for homogeneity of variances were once again employed to ensure that the data met the ANOVA test assumptions. The data were both normally distributed and the variances homogeneous, therefore the raw data was used in the analysis.

Differences in predation intensity among sites could have affected the interpretation of the mark-recapture data. Sites with high or low predation relative to other sites could have been interpreted as having falsely low (i.e., lobsters eaten were perceived as having left the site) or falsely high (i.e., more lobsters appear to have remained on the site) short-term residency. Therefore, to account for differences in relative predation among sites the lobster tethering data was analyzed with a 2 x 12 model-I repeated-measures ANOVA. The first factor of interest was sample date (the repeated-measures effect, 2 levels) and the second factor was the proportion of tethered lobsters killed over the two-day experiment at each site (n = 12). The tethering data were arcsine transformed to meet the ANOVA test assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test).

A repeated-measures ANCOVA was used to evaluate if the placement of the artificial structures had any impact on the proportion of diseased lobsters on each site. Again, the covariates of habitat structure and macroalgal cover met the assumption of homogeneity of slopes, but they were not significant and were thus removed from the analysis and the data re-analyzed as a 3 x 4 model-I repeated-measures ANOVA. The first factor in this analysis was treatment (high density enhanced, low density enhanced, and natural) and the second factor (the repeated-measures effect) was sampling date. The data met the ANOVA assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) after square root transformation.

C. Relationship between density and nutritional condition, short-term residency and disease:

Multiple regression analyses was used to determine if various measures of juvenile lobster population dynamics were related to lobster density or selected features of the hard-bottom nursery habitat irrespective of experimental treatment condition. Therefore, I evaluated in separate multiple regression analyses whether disease, nutritional condition (i.e., mean hemolymph refractive index) or short-term residency (i.e., proportion recaptured after 5 days) could be predicted by a combination of mean lobster density, habitat structure, or macroalgal coverage. The data from each survey were analyzed separately to avoid pseudo-replication (*sensu* Hurlbert 1984), as were the data for large (> 30mm CL) and small (< 30mm CL) juveniles because of ontogenetic changes in juvenile behavior, habitat use, and morphology that may influence population dynamics (see Butler & Herrnkind 2000, for review). In all regression analyses, the multiple regression assumption of multicollinearity was evaluated using multiple correlation analysis of the independent variables. None of the independent variables were correlated in the regression analyses performed on the individual surveys.

D. Relationship between density and nutritional condition, short-term residency and disease (all surveys combined):

The hemolymph refractive index, short-term residency, and disease regression data were further explored through plots and multiple regression analysis of the entire data set combined, to look for broad trends not evident in the subsets. This may violate the assumption of independence among observations because of the repeated temporal sampling at the same sites, however survey period was explicitly accounted for as an

independent variable. In all combined-survey multiple regression analyses data were linearized by $\log_{10}$ transformation. The multiple regression assumption of multicollinearity is discussed in the Results section.

Results

A. Dietary intake influence on nutritional condition:

The two refractometers used in the dietary intake study and nutritional condition measurements from the field studies were nearly identical (Fig. 20). The minor deviations in the measurements are probably due to errors in dilution preparation. Lobsters fed 100% of their daily intake had consistently higher serum protein values after six weeks than those fed 25% of their daily intake (Fig. 21, Table 5). To confirm that altering the feeding levels of the lobsters affected their condition, the CL and wet weight were measured upon initiation and termination of the experiment. This index has been shown to be an additional indication of lobster condition (Robertson et al. 2000, Oliver and MacDairmid 2001). While there was no effect of sampling date alone on the refractive index, both the feeding treatment and the feeding treatment * date interaction significantly affected the hemolymph refractive index (Table 5). The weight/CL ratio was not affected by the feeding treatment alone, but was significantly affected by the sampling date and the feeding treatment * date interaction.

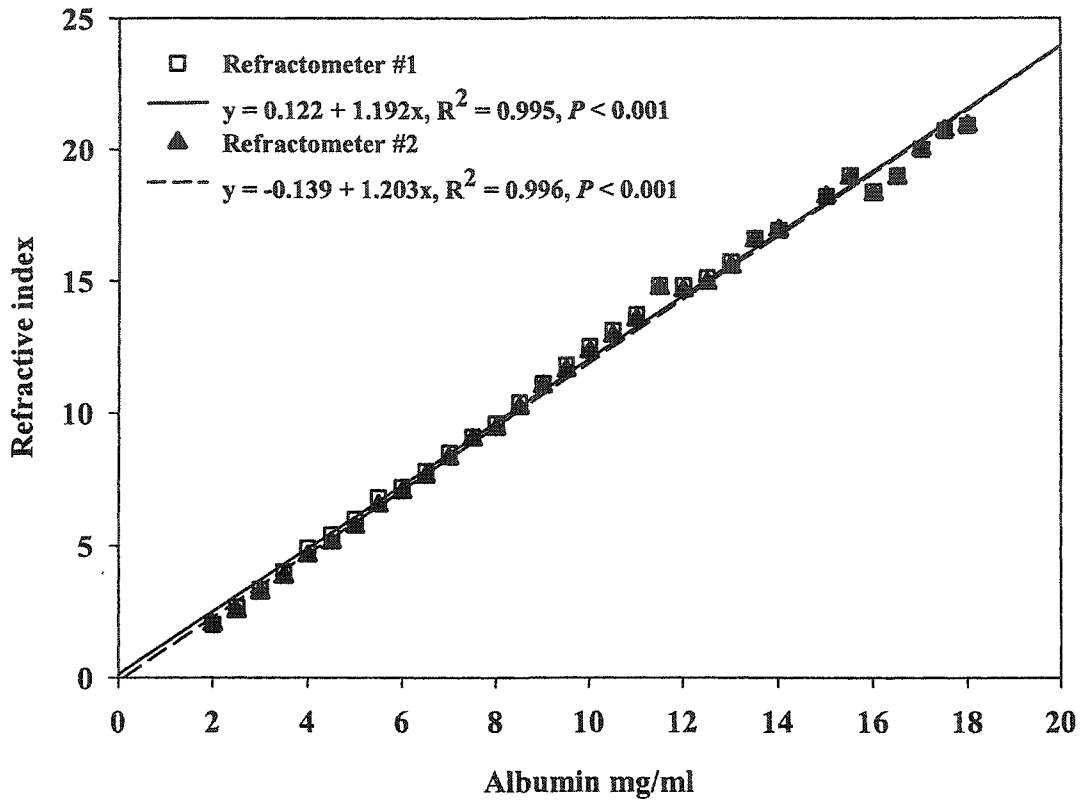

FIG. 20. Standard curve of refractive index against bovine albumin standard. Serial dilutions were made from an initial 30% albumin stock solution. Refractive indices were read on two identical Leica industrial refractometers.

TABLE 5. A 2 x 2 model-I repeated-measures ANOVA testing the effects of feeding level and experimental date on hemolymph refractive index and weight/CL ratio. "Date" represents the initiation and termination dates of the experiment when vital data were collected. "Feeding trt" represents the two treatment levels, 25% and 100%, of daily intake.

Source	Measure	df	Mean Square	*F*	*P*
Date	Refractive index	1	0.0113	0.040	0.843
	Weight/CL	1	0.0297	11.506	0.002*
Feeding trt	Refractive index	1	5.7980	19.247	<0.001*
	Weight/CL	1	0.0570	0.142	0.708
Date * Feeding trt	Refractive index	1	5.8590	20.759	<0.001*
	Weight/CL	1	0.0177	6.871	0.013*
Error	Refractive index	36	0.2820		
	Weight/CL	36	0.0005		

* significance determined at alpha = 0.05

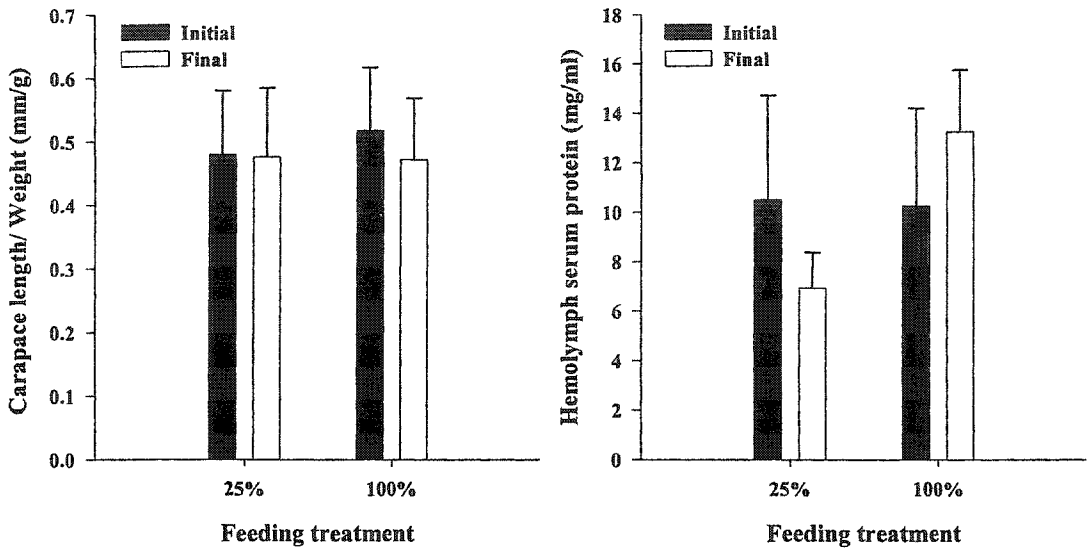

FIG. 21. Results of the dietary impact study comparing, on the left: the weight to CL ratio and on the right: hemolymph serum protein (mg/ml) for lobsters measured at the start of the study and after six weeks of feeding at 25 and 100% of their maximum daily consumption. Hemolymph refractive index was converted to serum protein using the equation: $y = -0.139 + 1.203x$, from Fig.20. Error bars represent 1 sd.

B. Impact of artificial enhancement on juvenile lobster population dynamics:

The density of juvenile lobsters was significantly higher at field sites where I enhanced shelter availability with many artificial shelters (25 - 50 shelters), as opposed to just a few (12 -18 shelters), confirming my ability to artificially manipulate lobster density in those treatments (Table 6). However, lobster density varied considerably among natural, unmanipulated sites. Therefore lobster density in this treatment did not differ significantly from the two treatments where shelter was manipulated (Fig. 22). If the experiment-wise error rate is used ($0.05/5 = 0.010$), and five separate ANOVAs are performed on this same data set, the significance becomes borderline. Shelter treatment had no discernable affect on the mean hemolymph refractive index of lobsters per site nor did it have a significant affect on the short-term residency of lobsters per site (Table 7). Relative predation did not differ among sites in the percentage of lobsters surviving the

two-day tethering experiment ($F_{1,11} = 2.092$, $P = 0.118$). Since relative predation intensity did not differ among sites, it was therefore assumed not to be a factor in the measure of short-term residency.

TABLE 6. A 3 x 4 model-I repeated-measures ANOVA on lobster density among site treatments, high density manipulated (HDM), low density manipulated (LDM), and natural (NAT) (unmanipulated). The results of the LSD multiple comparison test are shown below the ANOVA table. Treatment group means that share an underline are not significantly different from one another.

Source	df	Mean Square	*F*	*P*
Treatment	2	348.839	6.469	0.023*
Survey date	3	143.022	2.813	0.125
Treatment * Survey	6	49.881	0.360	0.898
Error	28	138.375		

* significance determined at alpha = 0.05

LSD multiple comparison results:	LDM	NAT	HDM
Means:	13.08	21.64	22.79

TABLE 7. Two separate 3 x 4 model-I repeated-measures ANOVAs examining the effect of habitat treatment on the mean hemolymph refractive index and short-term residency of lobsters/site.

Source	df	Mean Square	*F*	*P*
Refractive index				
Treatment	2	1.185	0.471	0.644
Survey date	3	5.173	2.040	0.208
Treatment * Survey	6	2.541	1.255	0.309
Error	28	2.024		
Residency				
Treatment	2	0.2640	2.973	0.109
Survey date	3	0.0074	0.077	0.970
Treatment * Survey	6	0.0991	1.886	0.120
Habitat availability (covariate)	1	0.4490	8.538	0.007
Error	27	0.0525		

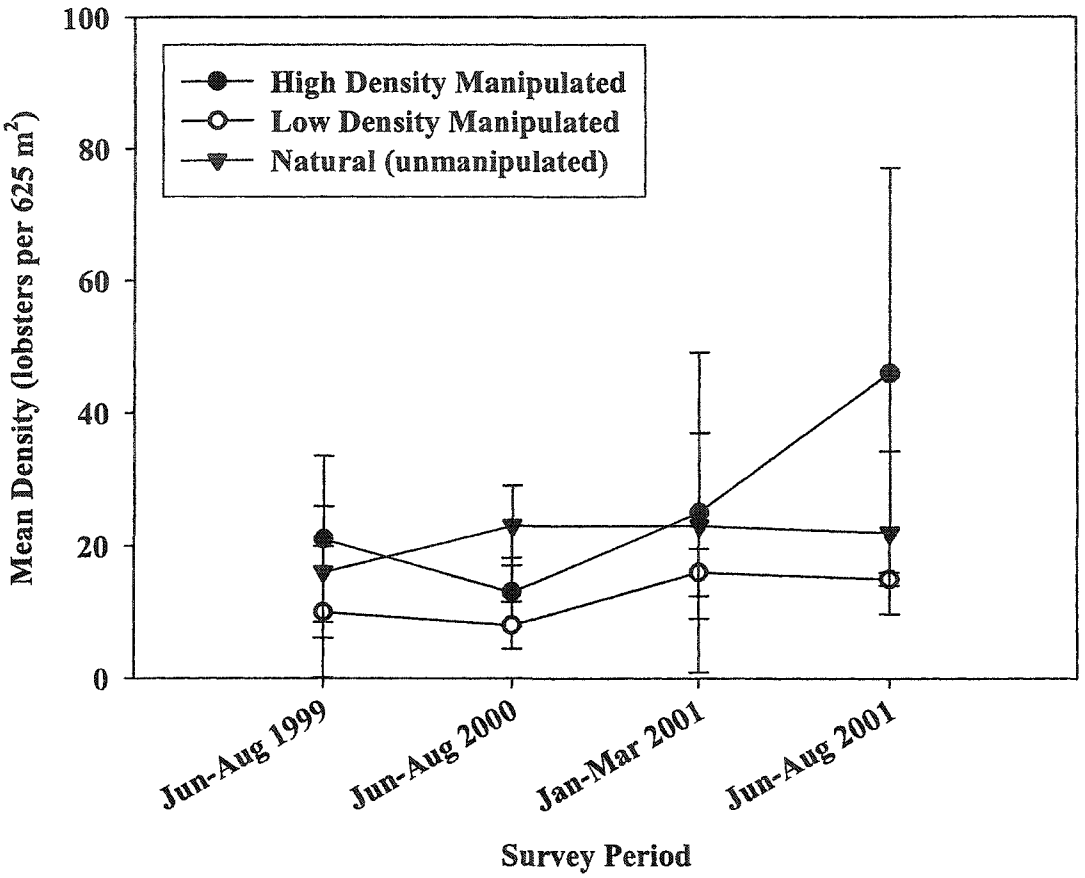

FIG. 22. Lobster density as a function of shelter treatment (HDM = high density manipulated, LDM = low density manipulated, and NAT = natural non-manipulated) for each survey. Error bars represent 1 SD.

The proportion of lobsters infected with the HLV-PA virus was not affected by habitat treatment, though there was a significant effect of survey date (Table 8.). The significant difference in HLV-PA prevalence among survey dates was due to differences in the number of infected lobsters sampled in each survey.

TABLE 8. A 3 x 4 model-I repeated-measures ANOVA examining the impact of artificial habitat enhancement on the prevalence of diseased lobsters per site. Habitat type represents the two general treatments evaluated here, artificially enhanced and natural.

Source	df	Mean Square	*F*	*P*
Habitat type	2	0.0425	2.421	0.154
Survey date	3	0.1460	8.728	0.012*
Habitat type * Survey	6	0.0164	0.395	0.876
Error	28	0.0415		

* significance determined at alpha = 0.05

C. Relationship between density and nutritional condition, short-term residency and disease:

Small (<30 mm CL) lobster density showed no correlation with mean hemolymph refractive index in any of the surveys, though three regressions between lobster density and nutritional condition were significant for large (>30 mm CL) lobsters during the three summer surveys (1999, 2000 and 2001) (Fig. 23, Table 9). However the independent variables (i.e., density, macroalgal cover or habitat structure) included in the significant models differed among regressions. Furthermore, the only variable that was significant in all three equations, habitat structure, was positively correlated in the June-August 1999 and 2001 surveys, but negatively correlated in the June-August 2000 survey. To explore whether a potential relationship was obscured by the low sample size of the separate survey data sets, I also plotted refractive index against density for the entire data set and re-analyzed the combined data set. There was still no significant relationship between nutritional condition and density, habitat structure or macroalgal cover for either small or large juvenile lobsters.

TABLE 9. Eight separate multiple regression analyses examining the relationship between mean hemolymph refractive index of small (<30mm CL) and large (>30mm CL) juvenile lobster over the 5-day mark-recapture and lobster density, habitat structure and macroalgal cover.

Survey period	Adjusted R^2	Mean Square	df	*F*	*P*
Small lobsters (<30mm CL)					
June-August 1999	-0.178	2.015	3	0.698	0.612
June-August 2000	0.537	4.600	3	4.096	0.082
January-March 2001	-0.184	3.452	3	0.431	0.737
June-August 2001	-0.164	4.187	3	0.484	0.703
Large lobsters (>30mm CL)					
June-August 1999	0.930	10.488	3	27.514	0.011*
Model: Refractive index = 4.786 + 0.108(density) + 8.918(cover) + 0.316(structure)					
June-August 2000	0.648	5.917	3	5.910	0.042*
Model: Refractive index = 11.90 + 0.099(density) - 0.283(structure)					
January-March 2001	0.188	6.916	3	1.851	0.216
June-August 2001	0.766	7.734	3	12.975	0.002*
Model: Refractive index = 4.786 + 8.918(cover) + 0.316(structure)					

* significance determined at alpha = 0.05

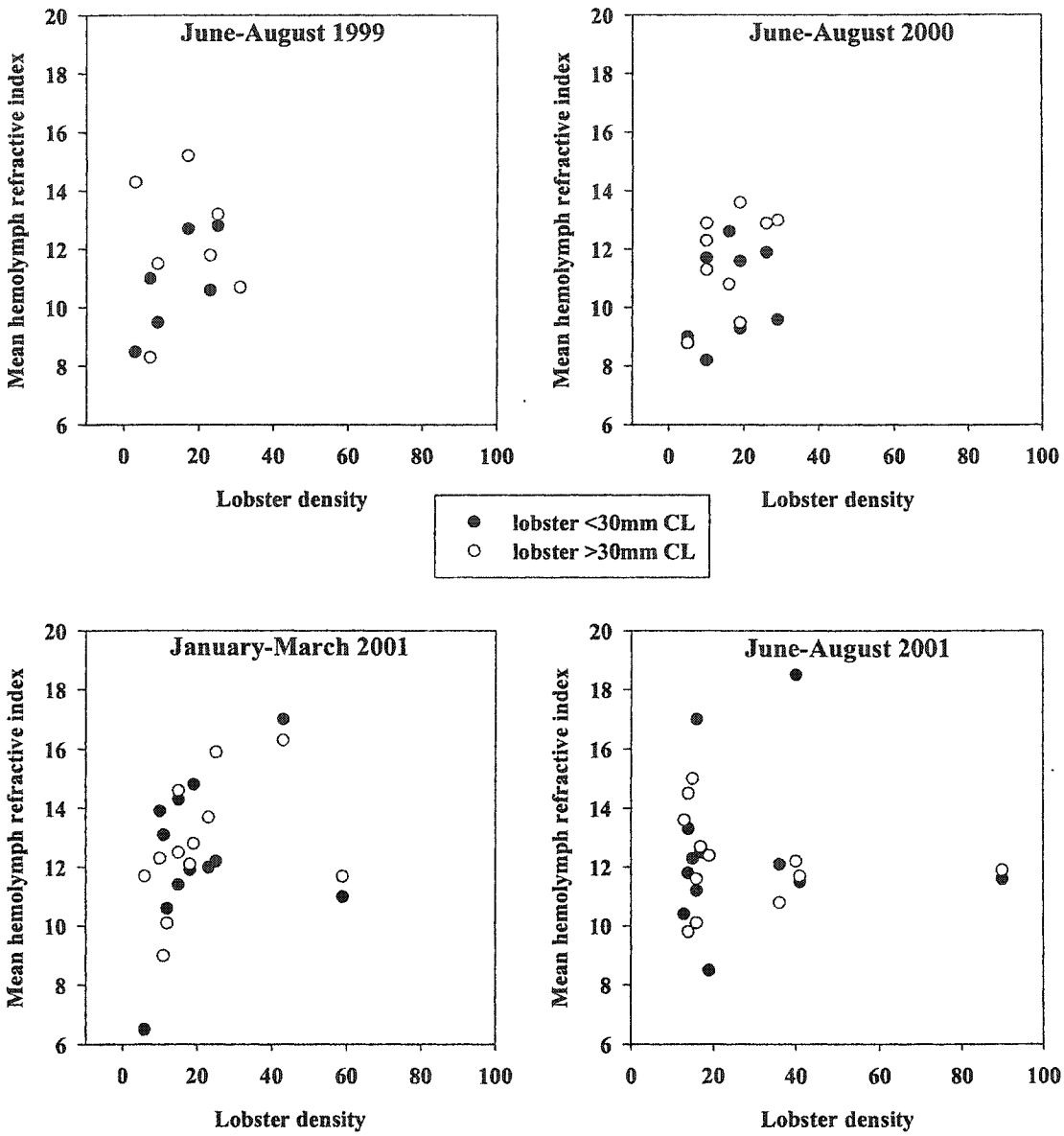

FIG. 23. Mean hemolymph refractive index as a function of lobster density for each of the four survey periods.

The proportion of lobsters, either small (<30 mm CL) or large (>30 mm CL), recaptured on a site at the end of a 5-day mark-recapture was also unrelated to density (Fig. 24) or the environmental characteristics of habitat structure or macroalgal cover during any survey period (Table 10). The regression models developed for small lobsters for the summer 1999, summer 2000, and summer 2001 regressions were of borderline

significance, however, the variables were not consistent among models, nor were the directions of the relationships. Since no pattern was apparent, I therefore assumed the borderline significance was due to the small sample size in each survey.

TABLE 10. Eight separate multiple regression analyses examining the relationship between short-term residency of small (<30 mm CL) and large (>30 mm CL) juvenile lobster over the 5-day mark-recapture and lobster density, habitat structure and macroalgal cover. No significant regressions were developed.

Survey period	Adjusted R^2	Mean Square	df	*F*	*P*
Small lobsters (<30 mm CL)					
June-August 1999	0.720	0.110	3	6.190	0.084
June-August 2000	0.564	0.130	3	4.454	0.071
January-March 2001	-0.031	0.085	3	0.889	0.487
June-August 2001	0.451	0.101	3	4.018	0.051
Large lobsters (>30 mm CL)					
June-August 1999	0.462	0.102	3	2.719	0.217
June-August 2000	0.333	0.084	3	2.331	0.191
January-March 2001	0.290	0.097	3	2.498	0.134
June-August 2001	0.182	0.095	3	1.818	0.222

There was no relationship between lobster density, habitat structure, or macroalgal cover and the prevalence of the HLV-PA disease at field sites as determined by multiple regression analysis (Table 11). I further analyzed the prevalence and density data separately for two size classes of lobsters: small (< 30 mm CL) and large (> 30 mm CL) (Fig. 25). I did so because smaller lobsters were more susceptible to infection by HLV-PA (see Chapter III) and the presence of the larger lobsters in the analysis may have obscured a density relationship among smaller lobsters. No relationship was observed with separate multiple regression analysis. Furthermore, removal of outliers did not improve the relationship.

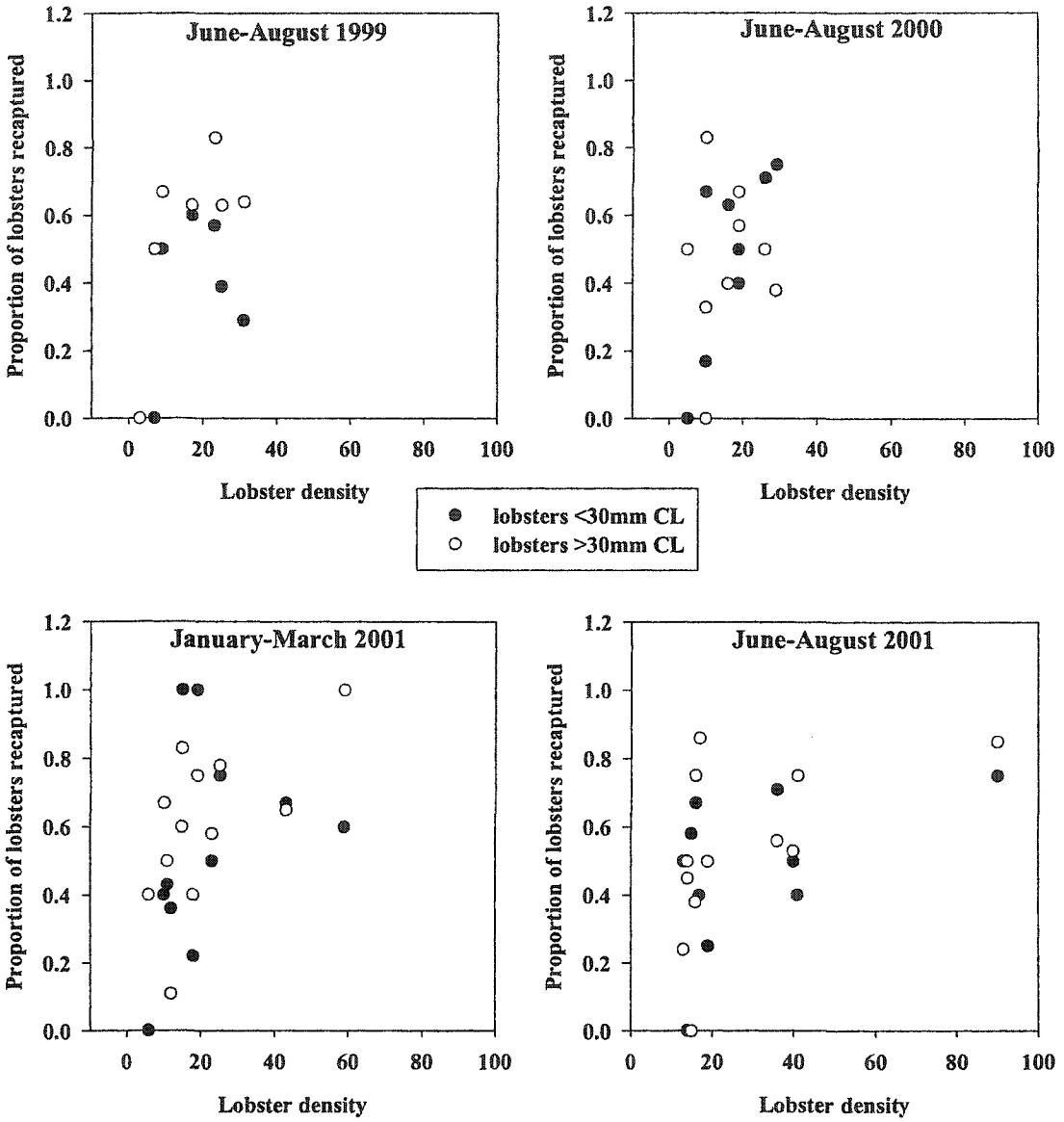

FIG. 24. Short-term residency of small and large juvenile lobsters over a 5-day mark-recapture as a function of lobster density per site for the four survey periods.

TABLE 11. Four separate multiple regression analyses examining the relationship between HLV-PA prevalence and lobster density, habitat structure and macroalgal cover.

Survey period	Adjusted R^2	Mean Square	df	*F*	*P*
June-August 1999	-0.450	0.0304	3	0.380	0.776
June-August 2000	0.092	0.0076	3	1.271	0.379
January-March 2001	0.316	0.0071	3	1.200	0.946
June-August 2001	0.150	0.0088	3	1.055	0.420

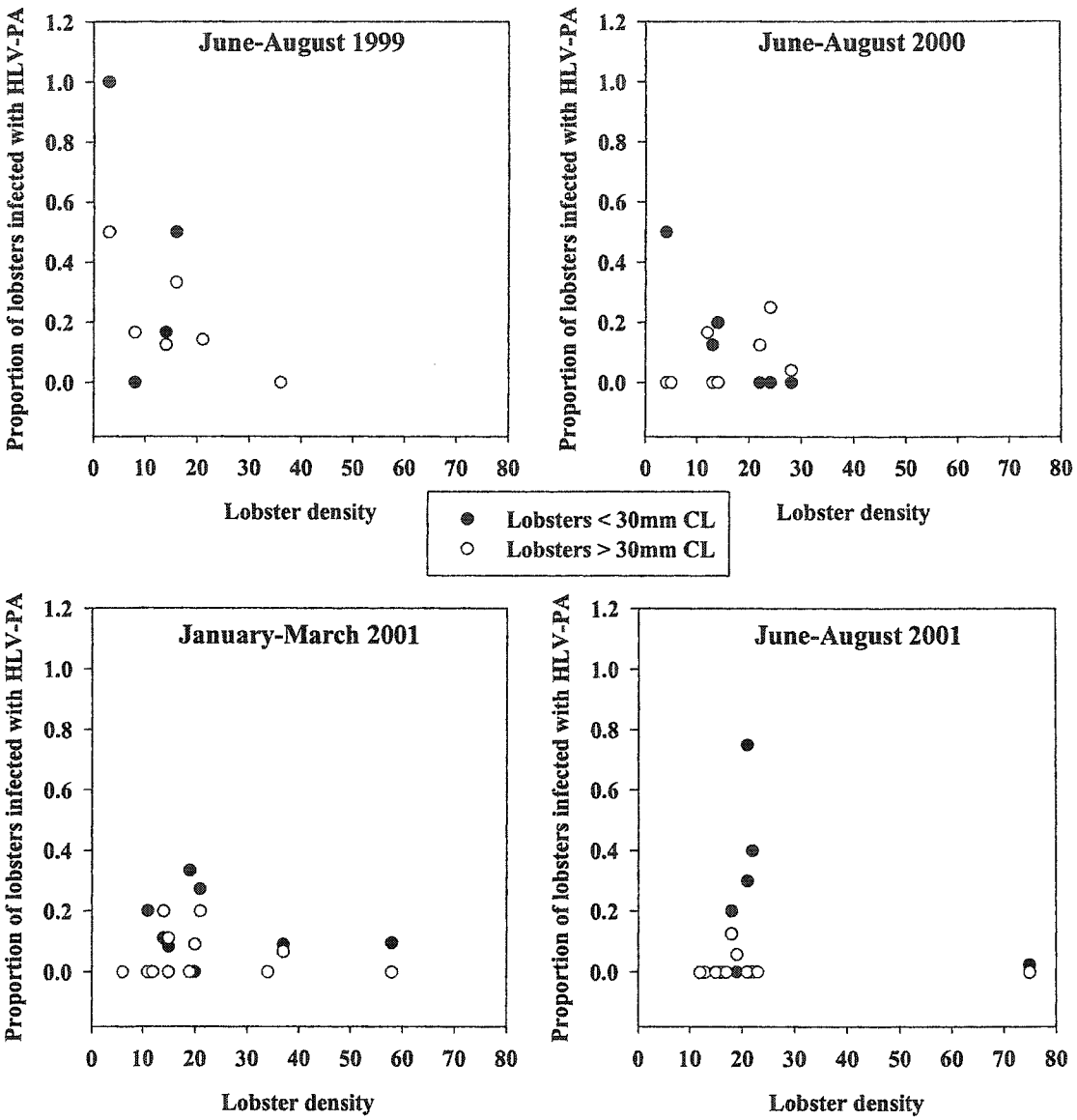

FIG. 25. Prevalence of HLV-PA infection in small and large juvenile lobsters as a function of lobster density per site for the four survey periods.

D. Relationship between density and nutritional condition, short-term residency and disease (all surveys combined):

These investigations, like the independent analyses conducted for each survey date, also revealed no relationship between the independent variables: lobster density, habitat structure, macroalgal cover and survey date and the dependent variables:

hemolymph refractive index or the incidence of HLV-PA viral infection. I therefore excluded these last two non-significant analyses from presentation here.

For the combined data, as the density of lobsters per site increased, the proportion of both small and large lobsters recaptured increased (Fig. 26). The independent variables of macroalgal cover, habitat structure and survey date were not significant predictors for recapture proportion among the small size-class lobsters and were therefore not included in the regression equation. Similarly, neither habitat structure nor survey date were significant predictors of recapture proportion among the large size-class lobsters. Macroalgal cover was significant in the regression for large lobsters, but was also correlated with density (Pearson correlation = 0.235, n = 80, $P = 0.036$). Density was the stronger predictor ($P_{\text{density}} < 0.001$, $P_{\text{macroalgal cover}} = 0.011$) thus macroalgal cover was removed from the regression. The regression was not improved by eliminating the size-class division in the data.

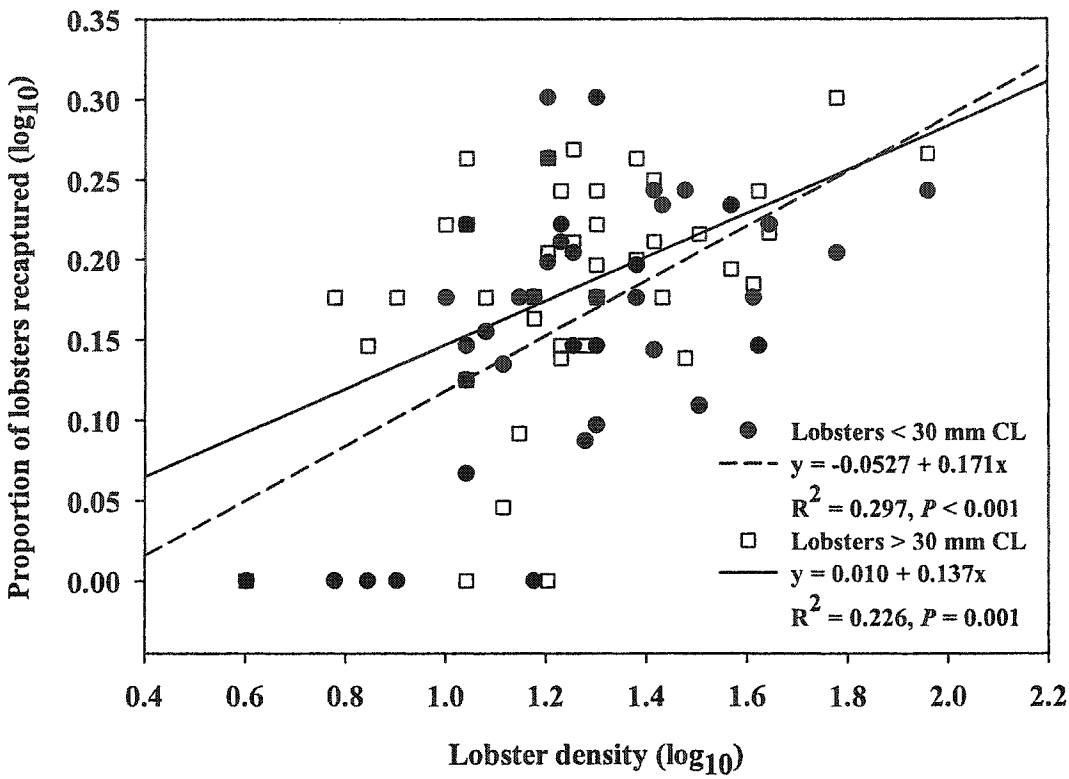

FIG. 26. Short-term residency of small and large juvenile lobsters over a 5-day mark-recapture as a function of lobster density per site for all surveys combined.

Discussion

Artificial enhancement of crevice shelters in natural nursery habitat successfully increased juvenile lobster densities, but differences in lobster density among mark-recapture sites had no measurable effect on lobster nutritional condition (i.e., hemolymph refractive index), local residency, or the prevalence of the herpes-like virus, HLV-PA. Similarly, in the independent surveys none of the variability in refractive index, residency, or disease prevalence could be explained by site characteristics such as lobster density, habitat structure or macroalgal cover. Significant relationships were found between hemolymph refractive index in large lobsters (> 30 mm CL) and these site

characteristics in the June-August surveys of 1999, 2000, and 2001, but the pattern of these relationships was inconsistent among surveys. When data from all survey periods were combined and reanalyzed a positive relationship emerged between lobster density and short-term residency, all other results remained unchanged.

Effects of artificial habitat enhancement

The benefits to recruitment of enhancing the availability of appropriately sized shelters as refuges from predators has been extensively tested in lobsters (Wahle and Steneck 1991, Mintz et al. 1994, Herrnkind et al. 1997*b*, Butler and Herrnkind 1997, Eggleston et al. 1990, 1992, 1997) and other decapod crustaceans. Appropriately sized shelters not only limits adult stone crab abundance (*Menippe mercenaria*), but also individual size and fecundity, because adult crabs seek shelter for both molting and egg production (Beck 1995, 1997). Populations of gonodactylid stomatopods may also be limited by habitat-mediated bottlenecks (Steger 1987). In aggregate, these and other studies have demonstrated that augmentation of the natural habitat with appropriately-placed and -sized structures results in increased abundance through greater survival of shelter-limited species, especially if these structures are targeted at the specific size or stage where the limitation occurs (see Beck 1997 for review of bottleneck hypothesis). I have demonstrated that artificial habitats increase the local density of juvenile Caribbean lobsters in Florida. But increased lobster density, whether accomplished by habitat enhancement or due to natural differences among sites, appears to have little effect on lobster nutritional condition or infection by the HLV-PA virus. Increased density has only a moderate influence on the short-term residency of larger juvenile lobsters.

This study was not directly designed to address the attraction-production issue, though it does yield some additional insight into the ramifications of enhancement. Lindberg (1997) outlined a series of questions aimed at evaluating the efficacy of artificial enhancement efforts. In short they are as follows: (1) "By what mechanisms or processes *might* artificial reefs enhance fish production?" (2) "Are any of these mechanisms or processes affected by characteristics of artificial reefs?" (3) Can the rates of processes, confirmed under question 2, be shifted favorably relative to control conditions?" (4) "If the answers to questions 2 and 3 are "yes," then is the gain in productivity or production sufficient to offset associated fishing mortality?" The final question does not apply to juvenile lobsters, which can not be legally harvested. To these questions I add the following: (5) are there non-lethal effects of artificial enhancement on the target population, and (6) what effect does enhancement of the target population have on the surrounding community?

A substantial amount of work has been done regarding the first three questions with respect to spiny lobsters. Butler and Herrnkind (1997) have demonstrated the existence of a demographic bottleneck, where small (< 35 mm CL) juvenile lobsters are habitat limited. Addition of artificial structures (identical to those used in the present study) substantially improved the survival and retention of small juveniles. Furthermore, they were able to discount immigration (attraction) through mark-recapture measures of short-term residency and post-algal survival, both of which were increased or enhanced relative to natural sites. Intuitively, the proper scaling of shelter dimensions to lobster size is critical, as this largely dictates the protection afforded therein (Spanier and Zimmer-Faust 1988, Eggleston et al. 1990, 1992 Eggleston and Lipcius 1992). The

artificial structures (i.e., concrete partition blocks) used in this study effectively replicate the size, crevice characteristics and distribution of shelters in the natural habitat (Butler and Herrnkind 1997). Much of the empirical work on the utility of concrete partition blocks in relieving the recruitment bottleneck have been positive, though caution is still advised in their employment (Butler and Herrnkind 1997). Systems throughout the Caribbean in which spiny lobsters reside can be markedly different (i.e., larval supply dynamics, settlement habitat and community composition) and thus their functionality and impacts on the surroundings may differ (e.g., Acosta and Butler 1997).

Concrete blocks are not the only structures that have been advocated for use in enhancing lobster stocks. Artificial "casitas", open-sided roofed structures of variable height, have long been used by fishermen throughout the Caribbean to concentrate lobsters for ease of capture (Cruz and Phillips 2000). Briones-Fourzan and Lozano-Alvarez (2001) employed these casitas to determine whether a demographic bottleneck might not also exist for *P. argus* juveniles in the lagoon of Puerto Morelos, Mexico. In this system, where larval supply is abundant, but juvenile lobsters scarce, the deployment of arrays of casitas both increased the abundance and size of lobsters relative to natural areas. The fact that casitas are scaled in size to attract large lobsters is the crux of the argument against the use of them to enhance recruitment of small juvenile lobsters. Casitas are, by design, much larger than most natural crevice shelters and as a result are not scaled to smaller (< 35 mm CL) lobsters. The protection afforded lobsters in casitas has also been shown to be dependent on the number of aggregated lobsters (Mintz et al. 1994). Furthermore, they tend to attract fish (Eggleston and Lipcius 1992, Eggleston et al. 1992, Mintz et al. 1994), many of which are lobster predators (e.g., snappers family

Lutjanidae, nurse sharks, *Ginglymostoma cirratum,* Smith and Herrnkind 1992, Eggleston et al. 1992; groupers family Serranidae, Schratweiser, M.S. Thesis, Old Dominion University, Norfolk, VA 23529). Beyond the potential drawbacks of casitas versus partition blocks as enhancement tools, the evidence that larger juvenile and adult lobsters are habitat limited is sparse.

While studies addressing the effectiveness of artificial enhancement measures abound, fewer studies have addressed question (4) above, and fewer still have examined the non-lethal population impacts and impacts on surrounding communities. Nizinski (Ph.D. Dissertation, The College of William and Mary, Gloucester Point, VA 23062), investigated the impact of lobsters and finfish on surrounding infaunal mollusc assemblages using casitas. No measurable affect on mollusc species richness or abundance was found, although size-specific predation by lobsters appeared to alter the size structure of gastropod populations near the casitas. As there are few studies addressing the impact of artificially enhanced *P. argus* on surrounding communities, I include examples from other organisms for comparison. The Dungeness crab (*Cancer magister*) has a significantly greater impact on the bivalve, *Macoma balthica*, in habitat enhanced with epibenthic shells compared to areas not enhanced with shells (Iribarne et al. 1995). Likewise, prey (i.e., adult echinoderms and molluscs) abundance increased significantly with distance from artificial reef units in a study of the effects of reef spacing on reef residents and surrounding foraging grounds (Frazer and Lindberg 1994). However, Ambrose and Anderson (1990) found, as Nizinski (Ph.D. Dissertation, The College of William and Mary, Gloucester Point, VA 23062) that the impact of artificial

reefs on surrounding infaunal organisms was limited to a narrow halo around the structure.

Results from the present study are also applicable to question (4) above, improving our understanding of the impacts of enhancement on sub-lethal population dynamics (i.e., nutritional condition, short-term residency and HLV-PA disease prevalence). Increased density through enhancement, at the level of this study, did not have a major affect on lobster condition or the prevalence of HLV-PA infection. It may actually increase site residency through the "guide effect" (see *Impacts on short-term residency*, below), which is hypothesized to aid lobsters in finding shelter and thus decreases predation (Childress and Herrnkind 1997, 2001). The gregarious nature of this mobile macroinvertebrate makes it robust to increases in density and lends it well to enhancement.

Partition blocks have the potential to greatly enhance lobster populations, but there is still much that is unknown such as: (i) at what density blocks themselves begin to affect the local environment (e.g., sediment, flow or boundary layer alterations) and its inhabitants, (ii) how and where they are most effectively placed (i.e., what environmental characteristics determine successful colonization?), (iii) the effective life-span of a block as a lobster shelter (i.e., when do they become filled with sediment or when do fouling organisms yield them inaccessible?), and (iv) how blocks operate in other systems with different physical (e.g., bathymetry, habitat characteristics, flow regimes) and biological dynamics (e.g., larval supply, post settlement mortality, community composition). There is data on many of these characteristics for other artificial enhancement techniques (e.g., Beets and Hixon 1994, Frazer and Lindberg 1994, Lozano-Alvarez et al. 1994), but the

method in which partition blocks were utilized here and by Butler and Herrnkind (1997) is unique and, thus, requires assessment in this mode.

Effect of density on disease, nutritional condition, and short-term residency of lobsters

In this study, hard-bottom habitat sites had vastly different lobster densities, with the highest occurring on sites augmented with artificial shelters (48-1440 lobsters/ hectare, sd = 256). The high end of this range is similar to those observed by others investigating enhancement with artificial "casitas" (1200 lobsters/ hectare, Lipcius and Eggleston, *personal communication*) and well exceeds the density reported for the natural habitat (454 lobsters/ hectare, Forcucci et al. 1994; 160 lobsters/ hectare, Butler and Herrnkind 1997).

A. Impacts on disease:

The lack of an impact of habitat enhancement on the proportion of lobsters infected with HLV-PA, or any significant correlation between lobster density and disease may be explained by the exceptional behavioral modifications brought on by exposure to HLV-PA infected individuals. I have shown that normally gregarious, healthy individuals will actively avoid sheltering with diseased conspecifics (see Chapter III for details). This avoidance could ameliorate the influence of density on transmission by maintaining a social barrier between healthy and infected individuals, given adequate shelter availability. Juvenile *P. argus* populations in Florida are shelter limited in some regions, whereas at others they appear to be limited by postlarval supply (Butler and Herrnkind 1997). Thus, the need to avoid infected conspecifics while still finding adequate shelter at sites where shelter is limited may in itself pose a risk, because juvenile

lobsters are highly vulnerable to predators (Smith and Herrnkind 1992, Herrnkind et al. 1997*b*). Where shelter is limited and HLV-PA prevalence high, a healthy lobster could spend an inordinate amount of time searching for shelter or be forced to reside in substandard shelter, both elevating the risk of predation or possibly increasing the chance of exposure to disease. One might expect that as the number of available shelters declines (with increasing disease prevalence), the chance that a lobster tagged on the first day of the survey might have emigrated from the site while searching for shelter during the subsequent days increases. No relationship was observed in this study between site residency and the number of available shelters or the proportion of lobsters on a site infected with HLV-PA, though this study was not explicitly designed to address search time. A mark-recapture may not be the appropriate measure to address this question as it is only a point measurement of time when a lobster is in a den, and there was no direct way to determine the time invested in the search for that shelter. Furthermore, the number of sites in this study that fit the criteria of high prevalence and low shelter availability were limited and may not have been sufficient to reveal this relationship with ANCOVA. A field study in which replicate sites are chosen with limited shelter, and disease prevalence is manipulated with tethered infected animals, would likely yield more convincing results, though introducing large numbers of laboratory infected lobsters into the field would be problematic. A more appropriate study might involve mesocosms with several shelter abundance treatments crossed with several diseased lobster prevalence treatments. The mesocosm would permit a more precise quantification of time spent searching versus time within a shelter.

B. Impacts on nutritional condition:

The efficacy of the use of hemolymph refractive index as a proxy for serum protein, and thus, nutritional condition was confirmed in my preliminary trials comparing the mean refractive index of lobsters fed 25% and 100% of their maximum daily consumption. Conversely the weight to CL ratio, though significantly different, did not reflect the treatments administered as expected. The weight/CL of the 25% group remained relatively constant, while the 100% group decreased. Though fed 100% of their maximum daily consumption, the change in diet from the field to laboratory food resulted in a decrease in this ratio, this may have been due to the conversion of water or sugars into fat or protein in preparation for molting. The absence of a decrease in weight/CL for the 25% food group may have been due to uptake of water concomitant with loss of tissue (Dall 1974). Both treatments may also have been affected by the error inherent in wet weight measurement. In any case, the resultant changes in hemolymph refractive index due to diet alteration were much stronger than reflected in weight/CL. The hemolymph refractive index results were also similar to those reported in a recent study of the effect of food supply on the hemolymph refractive index in southern rock lobster (*Jasus edwardsii)* that were starved, rationed (fed 10% of their maximum daily consumption) or fed 100% of their maximum daily consumption (Oliver and MacDairmid 2001). In both studies, where the differences in sustenance were substantial, hemolymph refractive index, as a proxy for serum protein, proved to be a strong and reliable measure of nutritional condition. Therefore mean hemolymph refractive index should be an adequate measure provided the difference in nutritional condition between the groups being tested is large.

The sensitivity of the measure is limited by the inability to distinguish between lobsters that have recently molted, but no longer possess pliable exoskeletons (i.e., late molt stage AB), and intermolt lobsters (i.e., molt stage C) (Lyle and MacDonald 1983). Protein levels reach a minimum during the postmolt period, though not indicative of starvation, and continue to rise throughout the intermolt period until they reach a maximum during the premolt period (Musgrove 2001). Other molt stages which affect the hemolymph serum protein concentration (i.e., premolt stages D_0-D_{3-4}) (Musgrove 2001, Oliver and MacDairmid 2001) are easily identified by pleopod molt staging (Lyle and MacDonald 1983), but postmolt/ early intermolt condition is not as unequivocal (Behringer, *personal observation*) and requires the presence of a pliable exoskeleton for confirmation (Musgrove 2001). This situation may potentially confound studies using hemolymph refractive index as a nutritional index. For example, if numerous lobsters sampled from a population are in late postmolt (i.e., the exoskeleton is no longer pliable and visibly indistinguishable from intermolt), the low mean refractive index measured for these lobsters may lead to the erroneous conclusion that the population is in a nutritionally poor state. In future studies, the inclusion of hemolymph pigment stage with hemolymph refractive index or serum protein measurements may better reflect true condition as color allows discrimination between early-, mid-, and late- intermolt (Musgrove 2001). Hemolymph color changes throughout the molt cycle and is highly correlated with molt stage (Musgrove 2001).

Juvenile spiny lobsters captured in the field, regardless of size, appear to have acquired adequate nutrition relative to lobsters in the dietary impact study, as reflected in their moderate serum protein values. The mean hemolymph serum protein for lobsters in

the field surveys (10.3 ± 3.8) was similar to the mean serum protein for all lobsters at the initiation of the dietary impact study (10.3 ± 4.0), and closer to the final serum protein for the 100% treatment (13.3 ± 2.5) than the 25% treatment (6.9 ± 1.5). The exceptionally high final value for the 100% group is probably a reflection of the copious high quality food administered in the experiment (i.e., squid and shrimp, *ad libitum*).

I hesitate to draw any general conclusions from the three significant regression models developed for the relationship between hemolymph refractive index and lobster density, crevice shelter abundance and macroalgal cover for large juvenile lobsters due to inconsistencies in the respective loadings of each variable in those models. All three models contain a different suite of independent variables. The only variable present in all models, habitat structure, is inconsistent in the direction of its relationship to hemolymph refractive index and the macroalgal cover variable was present in only two of the three significant regressions.

There are two prominent characteristics of the ecology of *P. argus* that may have influenced this result. Foremost, the mobility of spiny lobsters may permit them to forage far from their daytime refuges to areas not depleted by locally dense conspecifics. Few estimates of spiny lobster home range are available and virtually none are known for small juveniles. Large subadult and adult *P. argus* have been estimated to forage up to 300 m from their dens (Herrnkind et al. 1975, Cox et al. 1997), though this distance probably declines with size. Large *P. cygnus* juveniles, dwelling on uncrowded reefs off the coast of Australia, are estimated to move no more than 15 m over the course of a year (Chittleborough 1974), but possibly > 50 m on crowded reefs (Phillips and Joll 1984). Adult *Jasus edwardsii* off the coast of New Zealand move an estimated 41 m per night,

though this varies with reproductive season (MacDairmid et al. 1991). Therefore, even if these home range estimates are scaled back for smaller juveniles, they may still forage broadly enough to negate any effect of density in the shelter habitat. Secondly, the nonspecific diet of *P. argus* and the abundance of available prey items such as small bivalve and gastropod molluscs found in the ubiquitous red macroalgae of hard-bottom habitat (Andree 1981, Marx and Herrnkind 1985*b*, Herrnkind et al. 1988, Nizinski, Ph.D. Dissertation, The College of William and Mary, Gloucester Point, VA 23062) combined with the rapid recolonization of it following depletion (Butler et al. 1997) may present small juvenile lobsters with nearly limitless food.

C. Impacts on short-term residency:

The positive relationship between density and short-term residency (when all surveys were combined) supports the "guide effect" theory, where lobsters use the odor of conspecifics to locate shelter (Childress and Herrnkind 1997). The life history characteristic that sets spiny lobsters apart, and the probable explanation for this result, is their gregariousness. Aggregation of Caribbean spiny lobster is promoted through their use of the chemical cues to locate conspecifics (Ratchford and Eggleston 1998, 2000,Childress and Herrnkind 1996, Childress and Herrnkind 1997). This attraction is hypothesized to decrease the time spent searching for shelter because the likelihood that a conspecific is already in a suitable shelter is substantial (Childress and Herrnkind 1997, 2001).

The positive relationship found between short-term residency and density when all surveys were combined did not persist when surveys were analyzed separately. This is likely due to low replication during each individual survey but may have also been

affected by a confounding off-site factor. Because late stage juvenile *P. argus* are social, the presence of a large refuge such as large solution hole or commercial lobster trap near a site could influence local residency patterns. Although none of my sites contained large structures that might harbor aggregations of lobsters, the presence of such structures near a site (a situation that I occasionally observed), could result in abnormally high emigration from a site. Commercial lobster traps also concentrate lobsters and their effect would have been ephemeral, as traps are continually relocated during the season.

Conclusions

Both nutritional condition and the incidence of HLV-PA infection were independent of lobster density, natural or artificially enhanced. Paradoxically, short-term residency was positively influenced by density, irrespective of enhancement, as a consequence of the characteristic gregariousness of spiny lobsters. These results extend our understanding of the effects of artificial enhancement of this critical life stage. Though supportive of this method, further investigation is essential before their widespread use can be advocated. In conjunction, these results demonstrate the resistance of spiny lobsters to the detrimental affects of density and reinforce the benefits, both direct and indirect, garnered from sociality.

CHAPTER V

CONCLUSIONS

Although knowledge of the newly discovered HLV-PA pathogen is in its infancy, I have made strides in characterizing its structure, modes of transmission and its pathological consequences. This study has also brought to light some of the impacts that this pathogen has on lobster behavior and ecology. I have shown that transmission can occur by proximity or contact with an infected individual indicating that the avoidance of infected lobsters by their healthy conspecifics presumably reduces infection risk. To determine whether avoidance indeed reduces infection risk, the effective distance from an infected lobster and the time of exposure required for transmission need to be determined. Laboratory experiments investigating the effective transmission of HLV-PA over a series of distances are planned for 2003.

How healthy lobsters detect infected conspecifics is unknown, but positive results from the transmission studies and the proven chemosensory ability of spiny lobsters point toward chemical detection. Presuming chemical detection, isolation of the chemical that elicits an avoidance response could aid in determining the evolutionary relationship between spiny lobsters and HLV-PA. If the chemical to which healthy lobsters respond is specific to the virus, then an evolutionary adaptation specific to HLV-PA may have occurred. Conversely, if healthy lobsters are responding to a general chemical indicative only of poor condition, then avoidance behavior may be a more general response and could be triggered by other conditions resulting in poor health.

My initial studies on the modes of transmission of HLV-PA have been successful, though many avenues remain to be explored. Secondary host reservoirs, such as molluscan prey items or cohabitating decapods such as *Mithrax* spp. or *Menippe mercenaria* are a potential source for perpetuating HLV-PA if they become infected or act as carriers. The intriguing lack of infection in adult spiny lobsters, coupled with the significantly higher prevalence in progressively smaller size juveniles highlight the need for more research addressing whether this represents a shift in immunity with age or a habitat specific source of infection. Of course, the consequences of this disease for future lobster stocks also warrants further investigation. Fishery practices that may perpetuate the spread of HLV-PA among juvenile populations also need exploration. For example, in the Florida Keys fishermen are permitted to use juvenile lobsters as live social attractants (i.e., "bait") in their traps and are thus permitted to transport hundreds of juveniles in livewells onboard their vessels. It is possible that this practice may contribute to the confinement of diseased individuals with healthy ones, as well as the redistribution of diseased individuals throughout the Florida Keys. Excessive handling of juvenile lobsters by recreational or commercial fishermen may also injure them, which could render them more susceptible to disease. At present, we do not know if such practices influence the spread of the disease, but if so, we may be able to recommend measures to prevent further spread of HLV-PA. .

Similarly, an assessment of the occurrence of HLV-PA throughout the Caribbean is essential to identify regions where HLV-PA is present and those where it is not. This knowledge would aid in developing measures to isolate infected stocks.

Although contact/waterborne transmission is possible, HLV-PA prevalence does not appear to be associated with either natural or artificially enhanced lobster density. Furthermore, it appears that enhanced density has no measurable affect on juvenile Caribbean spiny lobster nutritional condition. The gregarious nature of spiny lobsters coupled with access to abundant prey items may make them robust to high density. Moreover, there was an unexpected positive impact of increased density on the short-term residency of juvenile lobsters, though in retrospect reasonable. The "guide effect" in which lobsters use the chemical odor of conspecifics to locate shelter is the most plausible explanation for local retention of lobsters where density is high.

Information regarding the use of artificial structures to augment juvenile lobster populations must be used with caution. Regions throughout the Caribbean in which spiny lobsters reside can be markedly different (i.e., larval supply dynamics, settlement habitat and community composition) and thus the functionality of artificial shelters and their impact on the surroundings may differ. Little is also known about the possible consequences of large-scale deployment of artificial structures on the natural communities, since nearly all studies have been limited in their spatial scope. The general use of artificial structures, to either mitigate for lost habitat or augment natural habitat, requires not only evaluation of the efficacy of their use, but investigations into the indirect impacts of their application.

In summary, these studies on HLV-PA form the initial groundwork for an understanding of a previously unknown virus, but much more remains to be done. The behavioral alterations brought about by HLV-PA are remarkable and warrant additional investigation into both their exact cause and ramifications (direct and indirect), for they

stand to alter our perceptions of the role of disease in structuring social populations.

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APPENDIX

Field Study Site GPS Coordinates

Site #	Treatment	Location	Latitude	Longitude
1	12 double blocks	Nearshore, bay, Grassy Key	N 24° 46.437	W 80° 57.091
2	50 double blocks	Grassy Key marker	N 24° 47.546	W 80° 57.738
3	Natural	Burnt Point	N 24° 45.656	W 80° 59.220
5	25 double blocks	Nearshore, South of Bamboo Key	N 24° 45.010	W 80° 59.755
7	23 double blocks	Between Burnt Point and Grassy Key marker	N 24° 47.208	W 80° 58.466
8	12 double blocks	~ 5km North of east end of Long Key Viaduct	N 24° 51.120	W 80° 52.201
10	50 double blocks	Channel Key Banks, ~7 km north of Long Key	N 24° 50.905	W 80° 53.699
11	Natural	KOA West	N/A	N/A
14	50 double blocks	Adjacent to east-side of Old Dan Bank	N 24° 50.487	W 80° 49.471
17	18 double blocks	Channel Key Banks, ~2 km North of Conch Key	N 24° 47.971	W 80° 54.406
20	Natural	Kemp Channel	N 24° 40.612	W 81° 28.546
21	Natural	Kemp Channel, near island closest to Blimp Road boat ramp	N/A	N/A

VITA

Donald C. Behringer, Jr.
Department of Biological Sciences,
Old Dominion University
Norfolk, VA 23529-0266 USA

Education

Current	Ecology Ph.D. program - Old Dominion University, Department of Biological Sciences, Norfolk, VA
B.S.	Zoology (Chemistry minor), 1991 - University of Florida, Biology Department, Gainesville, FL

Employment

1996-present	Graduate Teaching/Research Assistant, Department of Biological Sciences, Old Dominion University
1993-1994	Microbiologist, Quadrex Corporation/ BioEnergy International, Gainesville, FL
1992-1993	Microbiologist, American Bacteriological and Chemical Research Corporation, Gainesville, FL

Honors and Awards (last 4 years)

2002	National Shellfish Association Meeting, Student oral presentation competition, 2nd place
2002	Sigma Xi student poster competition, Old Dominion University, 2nd place
1998	Virginia Bagely Scholarship, Department of Biological Sciences, Old Dominion University
1998	Outstanding Graduate Teaching Assistant, Department of Biological Sciences nominee, Old Dominion University
1998	Phi Kappa Phi Honor Society, Old Dominion University