SHELL DISEASE IN IMPOUNDED POPULATIONS OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*, IN NOVA SCOTIA, CANADA

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ABSTRACT

The overall research goal was to identify risk factors for the development of shell disease in impounded populations of the American lobster, *Homarus americanus*. Shell disease, a disease syndrome characterized by progressive erosions and necrosis of the exoskeleton of aquatic crustaceans, continues to be one of the most prevalent and costly conditions affecting captive populations.

An epidemiological study was designed to evaluate the association between physiological and environmental factors and the development of shell disease on an individual lobster level. Initial health assessments on fresh caught post-harvest lobsters from Southwest Nova Scotia dealers showed significant differences between dealers, depending on the location of their respective fishing grounds (lower total protein levels, softer shells, and a higher proportion of intermolt lobsters in the primarily mid-shore dealer compared to the primarily inshore dealer).

Monthly observations of frequency and characteristics of shell disease lesions were performed during the storage period. Logistic regression results showed total protein as the strongest predictor of shell disease (odds ratios ranged from 4.3 to 32.8 for lobsters with low total protein levels, versus lobsters with high protein levels). The lobsters' molt stage also emerged as an important predictor for shell disease (odds ratios of 2.1 and 2.4 at models for 30 and 90 days storage) with a trend towards a relationship in the 60 and 120 day models, suggesting that the timing of the fishery in Southwest Nova Scotia in relation to the molt cycle is important in determining the post storage prevalence of shell disease in impoundment facilities. Shell hardness showed significance only in the 30 day model (odds ratio of 1.6), suggesting that this factor may only be important early in the storage period while sludge removal from the surface of the lobster was significant in 2 of the final models (odds ratios of 1.7 and 2.0 at 90 and 120 days, respectively), with a trend towards significance in the remaining two models, suggesting that the sludge offers some protective benefits to the captive lobsters.

From the perspective of a long term storage facility, the quality of the lobsters entering the facility is critical and, as this study shows, determines the extent to which shell disease will develop.

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CHAPTER 1

GENERAL INTRODUCTION

1 GENERAL INTRODUCTION

1.1 Atlantic Canada lobster industry

Over 100 years after its inception, the American lobster industry continues to be one of the most lucrative fisheries in coastal North America. In Atlantic Canada alone, the lobster harvest was valued at approximately $684 million in 2003 (DFO statistical service). With 9,700 licensed lobster fishermen and an export value of just over 1 billion Canadian dollars in 2003 (DFO statistical service), the industry maintains a critical socioeconomic role in rural fishing communities still struggling to survive after the collapse of the ground fishery.

While there are fears that this resource may meet with a similar fate, the lobster industry continues to be successful. This strength can be attributed to its unique characteristics including a luxury image, availability of global markets, geographical diversity with a wide range of fishing seasons spread over a wide area, and, most importantly, continued strong landings.

Despite these inherent strengths, today's lobster industry is still confronted with many of the same harvesting challenges as its predecessors over 100 years ago. This is not surprising, however, because many of the same traditional practices for long term holding, storage and transport are still being employed.

One of the most significant and problematic issues facing the industry is shell disease, a condition characterized by progressive erosions and necrosis of the exoskeleton of aquatic crustaceans (Rosen, 1970). Shell disease results in a

physically disfigured lobster, which is subsequently aesthetically unappealing to the consumer, and has reduced shipping abilities and elevated mortality.

1.2 Host species and geographic distribution

Shell disease was first described in the American lobster, *Homarus americanus*, in 1937 (Hess, 1937), and since then, the term has been used to describe various types of pathologic erosions in many species of *Crustacea* and geographical locations.

In addition to *Homarus americanus*, shell disease has also been reported in other species of lobster including the European lobster, *Homarus gammarus* (Roald et al. 1981), and the spiny lobster, *Panulirus spp. and Palinurus spp.* (Porter et al. 2001; Iverson & Beardsley, 1976).

Shell disease has also been affiliated with cultured prawn (*Macrobrachium rosenbergii*) populations from the United States (Brock, 1983) and the United Kingdom (El-Gama et al. 1986), and penaeid shrimp (*Penaeus spp.*) from the Gulf of Mexico (Cipriani et al. 1980; Cook & Lofton, 1973). Shell disease has also been reported in commercially harvested crab including blue crab, *Callinectes sapidus* (Rosen, 1967; Cook & Lofton, 1973), from the Gulf of Mexico to Chesapeake Bay, rock crab, *Cancer irroratus* (Young & Pearce, 1975), Dungeness crab, *Cancer magister* (Baross et al. 1978), and tanner crab, *Chionoectes tanneri* (Baross et al. 1978), from the eastern United States, as well as the Alaskan king crab, *Paralithodes camtschatica* (Follet & Grischkowsky, 1981).

1.3 Shell disease in *Homarus americanus*

The diversity in environments and species in which shell disease is observed suggests that the condition is ubiquitous (Getchell, 1989), and represents several distinct diseases manifesting themselves as one syndrome (Lightner and Lewis, 1975; Cipriani et al. 1980; Sinderman, 1989).

Within populations of the American lobster, three different types of shell disease are now recognized based on their respective pathology, etiology and epidemiology (Smolowitz et al. 1992). While this study focused on the impoundment form of shell disease, comparisons with the other types, burn spot lobster shell disease and epizootic lobster shell disease, were also presented.

Impoundment shell disease refers to lesions which develop during long term storage of live lobster within an impoundment facility (Smolowitz et al. 1992). Impoundment shell disease begins as bilaterally symmetrical lesions, centered around setal pores on the dorsum of the animal (Bullis, 1989). As the disease progresses, the eroded cuticle develops a "scooped out" appearance (Smolowitz et al. 1992). Despite being one of the best-described, impoundment shell disease continues to be the most prevalent, costly, and enigmatic pathologic conditions affecting impounded populations of the American lobster (Prince, 1997).

Burn spot (also referred to as rust or black spot) shell disease is characterized by individual circular blackened lesions at various locations on the body, and is generally associated with wild American lobsters populations in offshore canyons (Ziskowski et al. 1996).

The term epizootic lobster shell disease was first used in 2002 to refer to high levels of moderate to severe erosions observed in wild populations of the American lobster ranging from Eastern Long Island Sound to the nearshore waters of southeastern Massachusetts (Smolowitz et al. 2002). This form of shell disease is characterized by moderate to severe irregular, deep erosions along the dorsum of the carapace. Epizootic shell disease is believed to represent an extreme presentation of enzootic shell disease (Chistosterdov, 2005), which had previously existed at reduced levels in these regions for decades (Estrella, 1991).

1.4 Exoskeleton structure

The exoskeleton of crustaceans is a multi-layered tissue composed of cuticular and cellular components, which normally provides the animal with an effective barrier against disease. The exterior of the exoskeleton is the epicuticle, a very thin external layer composed of protein and calcium salts, but no chitin. By virtue of its composition, the epicuticle is considered to be biochemically inert, and protects the inner layers of the exoskeleton against solubilization of components, and invasion by microorganisms (Dennell, 1960; Stewart, 1980; O'Brien et al. 1993). Beneath the epicuticle lie three chitinous layers of the cuticle, commonly termed the exocuticle (calcified pigmented layer), calcified endocuticle and noncalcified endocuticle, followed by the membranous layer (Dennell, 1960; Aiken, 1980). The cellular portion of the exoskeleton includes the epidermis with associated glandular

structures and sensory receptors, responsible for secretion of all the non-cellular elements of the cuticle (Stevenson, 1985).

Functionally, the crustacean exoskeleton is considered to be a complex living tissue. The cuticle is permeated by numerous structures including channels or pore canals, ducts, and setae. Pore canals, believed to be active in cuticle synthesis and resorption, can extend through some or all layers of the cuticle, opening to the external surface of the epicuticle (Halcrow, 1993). Tegumental gland ducts also traverse the cuticle and are likely to have a secretory function - implicated in cuticle hardening, epicuticle formation and osmoregulation (Talbot and Demers, 1993). Setae refer to innervated cuticular hair-like sensillae which function in mechanoreception and chemoreception (Ache and Macmillan, 1980). Each seta consists of an external hair-like structure situated in a cuticular depression (setal pit), and a shaft which continues through the cuticle to a setal organ located below the epidermis.

1.5 Pathology of shell disease

Although many questions surrounding the etiology of shell disease in crustaceans remain, there is general agreement on the major aspects of its development. Typically, shell disease in lobsters begin as a series of small brown or black pits in the cuticle. As the disease progresses, these necrotic pits commonly extend laterally such that adjacent pits join to form larger areas of erosion (Rosen, 1967). Lesions are usually confined to the calcified layers of the integument, as the

non-calcified endocuticle and membranous layer appear to serve as a barrier to further microbial penetration (Rosen, 1967). However, the presence of microorganisms can confound the final stages of wound repair, leading to extensive destruction of the chitinous matrix, demineralization of the cuticle, and eventually resulting in perforation and penetration of the membranous layer, breaching the last line of epidermal host defense, leading to the ulcerative stage (Smolowitz et al. 1992). In aggressive manifestations of the syndrome, the exoskeleton of some appendages are completely eroded (Young, 1991), or branchial chambers are completely exposed (Engel and Noga, 1989).

Histologically, impoundment shell disease results in progressive areas of cleanly removed cuticle with little or no cuticular matrix left behind (Smolowitz et al. 1992). The presence of cuticle matrix was observed, however, in epizootic lobster shell disease lesions. In these lesions, "pillars" of lattice were observed projecting from the leading edge of the lesion, as a result of the replacement of the protein matrix between the lattice crystals by invading bacteria (Smolowitz et al. 2002).

1.6 Etiology of shell disease

At the present time, no specific single organism is considered the causative agent of shell disease. Burn spot shell disease has been primarily attributed to fungi (Rosen, 1970; Stewart, 1980) and/or bacteria (Rosen, 1970), while bacteria have been implicated as the principal agents in both impoundment and epizootic shell disease. Bacterial genera isolated from impoundment shell disease lesions include

Vibrio (Hess, 1937; Malloy, 1978), *Aeromonas* (Roald et al. 1981), and *Pseudomonas* (Malloy, 1978). While chitinolytic representatives of *Vibrio* spp. are routinely isolated from impoundment shell disease lesions as well as shell disease from several other species of *Crustacea*, they are only occasionally observed in epizootic lesions. Investigations by Chistoserdov et al. (2002; 2005) indicate that bacteria belonging to *Flavobacteriaceae* and *Pseudoalteromonas* are likely responsible for epizootic shell disease. The occurrence of "pillars" formed by the removal of matrix proteins and lipids suggests that the bacteria responsible for the development of epizootic lobster shell disease were not primarily chitin feeders, but were attracted to the matrix protein and lipids (Smolowitz et al. 2002).

The diversity of microorganisms associated with all forms of shell disease also suggests that there are multiple agents or mechanisms involved in the development of the disease syndrome. The shell lesion has been perceived as a microenvironment in which several microbial taxa produce a variety of degradative enzymes, which act over time to erode the cuticle (Rosen, 1970). In the microenvironment of a lesion, bacteria or fungi representing several taxa may interact to produce characteristic disease signs. Investigations with burn spot shell disease in both the edible crab, (Vogan and Rowley, 2002a) and shrimp (Cipriani et al. 1980) also concluded that shell disease was not caused by a single pathogen, but more likely the result of the collective effects of the lesion community.

Regardless of the host species or geographic location, there appears to be a general consensus emerging that the shell disease condition is an indication of a

'metabolic disturbance' that diminishes the host's normal defense mechanisms, such as chitin deposition and wound repair (Sindermann, 1991). This metabolic disturbance may be a consequence of physical damage to the shell, or environmental or physiological stressors acting as immunosuppressants (Bullis et al. 1988). The specific causes, however, of shell disease may be area-specific, varying according to the geographic location of the crustacean population (Vogan and Rowley, 2002b),

Unilateral lesions resulting from local metabolic disturbances of the exoskeleton may be caused by various mechanisms. The importance of physical damage or initial mechanical trauma to the epicuticle, in the development of shell disease has been demonstrated in various species including lobster (Malloy, 1978), shrimp (Dyrynda, 1998), and crab (Vogan et al. 1999; Cook and Lofton, 1973). Epicuticle penetration of the shell of crustaceans, however, may also occur through lipid hydrolysis by microbial enzymes on the exoskeleton without obvious physical damage (Baross et al. 1978; Cipriani et al. 1980). Unilateral lesions are usually less severe, with only the occasional perforation in severe cases of coalescing of adjacent areas.

Conversely, bilaterally symmetrical lesions derived from systemic metabolic disturbances are often severe in nature. In the dual pathways of disease described by Bullis et al. (1988), it was proposed that this 'endogenous' form of shell disease may be initiated by environmental exposures or poor physiological condition, which adversely impact the animal's metabolic process of chitin synthesis and shell repair. Therefore, in unhealthy or 'immunosuppressed' animals, these defense barriers may

be weakened, leading to access through the exoskeleton by microbes that would normally be kept out. As this form of shell disease develops, infected areas often coalesce into large confluent areas of pitted, heavily melanized cuticle as a consequence of the activity of chitin destroying microorganisms. This type of shell disease is initially associated with vulnerable sites such as setal pores, which are believed to be the area of lesion initiation in both impoundment and burn spot shell disease (Smolowitz et al. 1992; Malloy, 1978).

1.7 Risk factors for shell disease

Various environmental and physiological factors, either acting separately or together, have been implicated as possible 'immunosuppressors' in healthy crustaceans, presumably resulting in a rate of chitin deposition that is unable to keep pace with the activities of chitin degrading organisms (Sindermann, 1991). Environmental stressors that have been implicated include high organic loading of containment waters resulting in the multiplication of potentially pathogenic microorganisms (Sindermann, 1989), extreme temperature (Malloy, 1978; Stewart, 1980), reduced salinity, anoxia or hypoxia, and the presence of toxic chemicals or sewage (Young and Pearce, 1975; Gopalan and Young, 1975).

Toxic compounds have been implicated as facilitators to the development of shell disease in wild populations of lobster (*Homarus americanus*), crabs (*Cancer irroratus*) and shrimp (*Crangon septemspinosa*) captured in the severely polluted region of New York Bight (Young & Pearce, 1975; Gopalan &Young, 1975). These

investigators were also able to reproduce the shell disease erosions and ulcers in laboratory held, healthy lobsters exposed to seawater containing sewage sludge. Poor environmental quality could facilitate shell disease in crustaceans by increasing the chance of lesion formation due to the presence of higher levels and diversity of chitinolytic bacteria (Vogan and Rowley, 2002b) or due to a suppression of immune competence (Noga et al. 1994).

The effect of reduced temperature on lesion formation was implied by studies with impoundment shell disease in the American lobster (Malloy, 1978) which showed more infections were established in lobsters exposed to a chitinolytic bacterium at temperatures between 2 to 5°C than at 10 to 15°C. The higher prevalence of impoundment shell disease in winter pounded American lobsters as compared to spring or summer pounded populations (Hess, 1937; Taylor, 1948; Bullis, 1989) also suggests seawater temperature may be important in subsequent development of impoundment shell disease . Lobsters under winter conditions would presumably be less active metabolically and not be expected to have a high capacity for epicuticular repair (Stewart, 1980).

The effect of seawater temperature has also been suggested in the development of epizootic lobster shell disease, however, in this case, with increased prevalence of shell disease associated with thermal stress due to elevated summer water temperatures (Dove et al. 2004).

Molt stage has also been implicated as potential risk factor in both impoundment and epizootic lobster shell disease. In investigations with

impoundment shell disease, Malloy (1978) and Getchell (1991) observed that more disease was established in post-molt lobsters compared to pre-molt lobsters. Investigations with epizootic and enzootic lobster shell disease (Estrella, 1991; Castro and Angell, 2000) found that shell disease prevalence was significantly higher in larger lobsters, suggesting an inverse relationship with molting frequency and, therefore, size of the animal. It is believed that infrequent molting allows less opportunity for shell renewal following injury or invasion by chitinoclastic bacteria. Similar investigations also demonstrated higher disease prevalence in ovigerous females compared to males (Ziskowski et al. 1996; Young, 1991; Baross et al. 1978; Estrella,1991). Castro and Angell (2000) found the prevalence of shell disease in wild ovigerous females from Rhode Island to be over 50% compared to 10% in males, with the following suggested explanation: mature females carry their eggs for up to 9 to 11 months (Waddy et al. 1995) and molt with only one-half the frequency of males (Aiken, 1980) and thus, potentially retain diseased exoskeletons for longer periods. No information exists on the effect of size or reproductive condition on development of impoundment shell disease in lobsters.

The importance of proper nutrition in preventing impoundment shell disease in lobsters has been shown by researchers (Malloy, 1978; Fisher et al. 1976) who observed increased susceptibility to the disease in lobsters fed inadequate diets, presumably as a result of impaired host resistance. Similarly, in field trials in a lobster tidal pound, Prince et al. (1995) observed a decrease in shell disease and mortality in association with feeding of an energy dense, and nutritionally complete,

experimental diet, compared to the traditional diet of salt fish racks. It was suggested that the enhanced diet provided elements necessary for the processes of cuticular maintenance, wound repair, and internal defense mechanisms that are absent from the traditional feed.

Despite the fact that shell disease continues to be a problem in impounded or other captive populations of the American lobster, many of the potential risk factors implicated in the development of shell disease were identified from studies of epizootic or enzootic lobster shell disease in wild populations, and therefore may not be relevant to lobsters stored in an impoundment facility.

1.8 Shell disease and mortality

While mortality frequently accompanies shell disease in impoundment (Fisher et al. 1976) and burn spot shell disease (El-Gamal et al. 1986), the relationship is not well understood. In most cases, mortality appears to be the result of secondary infections, which occur as skeletal integrity is lost. Smolowitz et al. (1992) found the hemolymph of lobsters severely affected with impoundment shell disease contained motile, Gram negative bacteria as well as a ciliated protozoan. Death from impoundment shell disease has also been attributed to an impaired ability to respire caused by erosion and destruction of gill tissue (Sawyer and Taylor, 1949).

Investigations with shell disease in the edible crab (*Cancer pagurus*) showed a positive correlation between disease severity and the degree of infection of the

hemolymph with culturable bacteria (Vogan et al. 2001) leading researchers to suggest that the disease was not limited to external infections and may also coincide with low levels of systemic bacterial infections. Similar investigations with epizootic lobster shell disease, however, failed to show any concomitant internal infection, implying that the pathogenesis of this form of shell disease was strictly dermal (Chistoserdov et al. 2005).

1.9 Prevalence of shell disease

Accurate estimates of the prevalence of shell disease in impounded populations are difficult to obtain given the lack of sound scientific studies and the inherent difficulties in data collection in these facilities. Most impoundment facilities hold lobsters communally which makes it very difficult and, in many cases, impossible to obtain accurate records of disease incidence, mortality, or product history. In addition, there remains an inherent secretiveness and lack of communication within the industry that makes facilities, even with the proper inventory control system in place, very reluctant to release information. With respect to impounded American lobster populations, shell disease has been a significant problem for almost a century (Hess, 1937; Taylor, 1948). One survey of tidal pound owners in Maine indicated that up to 40% of pounds had previously experienced the condition following long term storage (Getchell, 1991).

We have observed that shell disease is a recurring problem in a lobster pound facility in Nova Scotia, Canada. The prevalence of disease peaks during the winter

season, and since 1996 has ranged between 0.1% to 2% (Theriault, unpublished observations). Despite relatively controlled environmental and storage conditions employed within a long term lobster storage facility, prevalences from specific sources of lobsters may still reach over 5%, translating into an annual cost of several hundreds of thousands of dollars due to their aesthetically unappealing appearance, drastically reduced abilities to withstand shipping and storage, and elevated mortality. However, little is known about the risk factors associated with the development of shell disease in an impoundment facility.

1.10 Research objectives and specific aims

The main goal of this study was to evaluate the association between various physiological and environmental factors and the development of shell disease in captive or impounded populations of the American lobster. Conducted within the controlled environment of a long term lobster pound storage system in Nova Scotia, Canada, this study presented a unique opportunity to apply the principles of epidemiology to impounded populations of the American lobster.

The specific aim of this study was:

To design an epidemiological study to examine the associations at the individual lobster level among dealers, sludge accumulation, pre-storage lobster parameters (total protein, shell hardness, sex, and molt stage), and shell disease development during storage in a lobster pound facility. Factors associated with shell disease were modeled at each of 4 times points (30, 60,

90, and 120 days storage) to determine the consistency with which associations could be found.

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CHAPTER 2

RISK FACTORS FOR THE DEVELOPMENT OF SHELL DISEASE IN IMPOUNDED POPULATIONS OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

2 RISK FACTORS FOR THE DEVELOPMENT OF SHELL DISEASE IN IMPOUNDED POPULATIONS OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

2.1 Abstract

Shell disease, a disease syndrome characterized by progressive erosions and necrosis of the exoskeleton of aquatic crustaceans, continues to be one of the most prevalent and costly conditions affecting captive populations of the American lobster, *Homarus americanus*. This study was designed to evaluate the association between various physiological and environmental factors to the development of shell disease in a long term lobster storage facility. A total of 540 lobsters free of shell disease upon arrival were randomly selected for testing for pre-storage laboratory parameters, and then examined for signs of shell disease at 30, 60, 90 and 120 days after storage. Factors associated with shell disease were modeled at each time point to determine the consistency with which associations could be found.

Significant differences in initial health assessment test results were observed between dealers, with lobsters from the primarily mid-shore dealer exhibiting significantly lower total protein levels (44.4 mg/ml compared to 62.7 mg/ml), softer shells (87 compared to 89 durometer units), and a higher percentage in intermolt (80.2% compared to 64.3%). Observation of the pattern of shell disease lesion distribution showed a bilaterally symmetrical appearance in 70.1% of the infected animals. Shell disease occurred with high cumulative incidences (ranging from 25.7% at 30 days storage to 52.4% at 120 days storage) in the absence of obvious physical damage to the shell.

Logistic regression analysis results showed total protein as the strongest predictor of shell disease in winter caught lobsters from Southwest Nova Scotia. The odds of developing shell disease were 4.3 to 32.8 times higher for lobsters with low total protein levels versus lobsters with high protein levels. The lobsters' molt stage emerged as an important predictor for shell disease in the 30 and 90 day models (odds ratios of 2.1 and 2.4, respectively), with a trend towards a relationship in the 60 and 120 day models. Shell hardness showed significance only in the 30 day model (odds ratio of 1.6), suggesting that this factor may only be important early in the storage period. Sludge removal from the surface of the lobster was significant in 2 of the final models (odds ratios of 1.7 and 2.0 at 90 and 120 days, respectively), with a trend towards significance in the remaining two models, suggesting that the sludge offers some protective benefits to the captive lobsters.

From the perspective of a long term storage facility, the quality of the lobsters entering the facility is critical and, as this study shows, it determines the extent to which shell disease will develop.

2.2 Introduction

The American lobster industry continues to be one of the most lucrative fisheries in coastal North America. While many improvements in image, marketing, technology and science have been introduced in its 100 year history, the industry today is still faced with some of the same storage issues as its predecessors. One of the most prevalent and costly pathologic conditions affecting impounded

populations of the American lobster continues to be shell disease. Shell disease is a condition commonly defined as a disease syndrome characterized by progressive erosions and necrosis of the exoskeleton of aquatic crustaceans (Rosen, 1970). Shell disease results in an aesthetically disfigured lobster that is subsequently unappealing to the consumer and has reduced shipping and storage ability.

While the cause of shell disease is still unknown, the last 10 years have seen a renewed interest due to its increased prevalence in wild caught lobsters from eastern United States (Glenn and Pugh, 2005; Castro and Angell, 2000). Recent research has identified this epizootic form of shell disease as being different from impoundment shell disease, which occurs during long term storage in impoundment facilities (Smolowitz et al. 1992).

Impoundment shell disease meets the criteria for the endogenous form of shell disease as originally described by Bullis (1989). This form of the disease was considered to be a result of a metabolic disturbance that diminishes the host's normal defense mechanisms (Sinderman, 1991). This metabolic disturbance may be a consequence of physical damage to the shell, which results in unilateral lesions, or a consequence of environmental or physiological stressors, resulting in bilaterally symmetrical lesions.

Several variables have been implicated as possible immunosuppressors in healthy crustaceans. Poor environmental quality could facilitate shell disease though a number of mechanisms. Higher levels and diversity of chitinolytic bacteria may increase the chance of lesion formation (Vogan and Rowley, 2002) while reduced

environmental quality could also result in suppression of immune competence in crustaceans (Noga et al. 1994). Physiological factors including molt stage (Malloy, 1978; Getchell, 1989; Goarant et al. 2000), nutritional status (Fisher et al. 1976; Prince et al. 1995), sex (Kapareiko et al. 2001), and size (Estrella, 1991; Young, 1991; Baross et al. 1978) have also been implicated as potential risk factors for shell disease.

The overall objective of this study was to examine, at the individual lobster level, the associations between various physiological parameters and shell disease development in impounded populations of the American lobster using statistical models. Factors examined in this study included total protein, shell hardness, molt stage, sex, dealer, and sludge accumulation.

2.3 Materials and methods

2.3.1 Lobster selection

This longitudinal study was conducted during the 2002 winter pounding season (November 2001 to April 2002) at a lobster pound in Arichat, Nova Scotia. Based on historical differences in shell disease prevalence, lobsters from two dealers located in Lobster Fishing Area (LFA) 34 in Southwest Nova Scotia were included in the study. Both dealers had been regular suppliers to the facility for over a decade. Although located in close proximity to one another, there were differences in their respective fishing grounds. The boats from one dealer covered more of the midshore grounds (inside the offshore LFA 41 line west of Browns Bank where depths are

greater than 50 m). The boats from the other dealer stayed primarily in the traditional nearshore fishing areas of LFA 34 (less than 50m) (Figure 2.1).

Between December 4 and December 14, 2001, study lobsters were selected from shipments of lobsters obtained from these 2 dealers during the grading process at the facility, as described below. During the grading process, all lobsters deemed suitable for storage (strong, undamaged, and equal sized claws), were placed on a conveyor belt, weighed individually, and then sorted by weight into separate bins. Only lobsters weighing between 1.00 and 1.15 pounds were eligible for selection. This weight range is referred to as "small chix". All lobsters included in this study were fresh caught (received at facility within 2 days of harvesting) as is typical for the industry. Each lobster was carefully inspected to confirm that it was uninjured and strong, and to ensure that only shell disease free animals (initially) were entered into the study.

From this sampling frame that met the above inclusion criteria, a study population of small chix was systematically randomly chosen at regularly spaced time intervals. Sampling was performed for approximately 4 hours per shipment, at a rate of approximately 36 animals per hour for 2 consecutive shipments for each dealer. In total, sample sizes of 288 lobsters from dealer 1 (primarily midshore), and 252 lobsters from dealer 2 (primarily nearshore) were obtained.

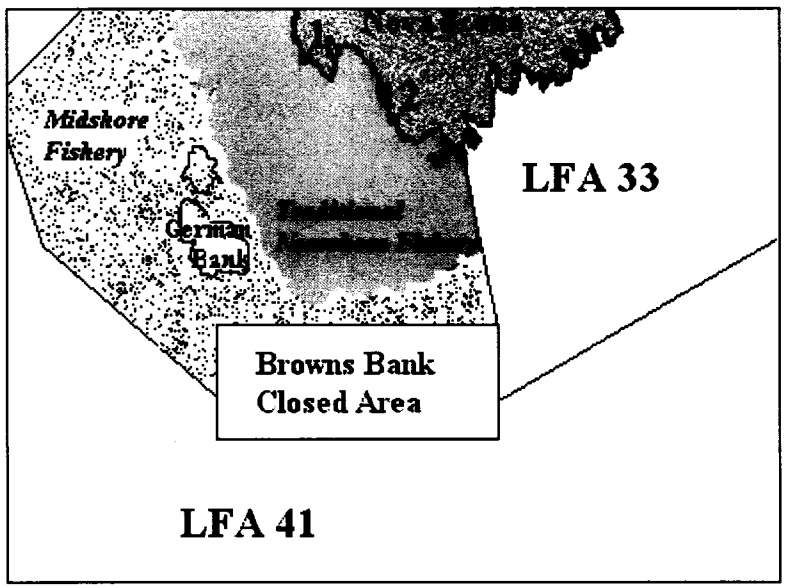


Figure 2.1. A map of LFA 34 and LFA 41 traditional nearshore and midshore lobster fishing areas indicating the location of the primarily midshore (1) and primarily inshore (2) dealer locations (modified from DFO, 2000).

2.3.2 Initial lobster health assessment

Sex, hemolymph total protein, shell hardness, and molt stage determinations were performed for each lobster included in the study. The sex of the lobster was determined through external examination of differences in the first pair of pleopods (Phillips et al. 1980). The first pleopods of the male are slender and rigid, while the first pleopods of the female are similar to the others but greatly reduced in size. Carapace length (CL), recorded in millimeters, was measured from the caudal end of the eye socket to the caudal extremity of the carapace.

To measure hemolymph total protein levels, hemolymph samples of 0.1 ml were removed from the ventral sinus of each lobster using a 1 ml syringe and 26-gauge needle. Samples were placed on a Atago temperature compensated refractometer for direct reading of refractive index. The refractometers were re-calibrated approximately every 30 minutes. Total protein (mg/ml) was calculated from the refractive indices based on the modified biuret method (Layne, 1957) using bovine serum albumin as the standard. Shell hardness (or carapace rigidity) was measured using a durometer (Hicks & Johnson, 1987; Foyle et al. 1989).

Molt staging was determined using both the setal staging and shell hardness methods. Setal development was used to identify premolt lobsters while shell hardness was used to distinguish late postmolt from intermolt lobsters (Table 2.1). Setal staging was conducted using the terminal one-quarter of the pleopod exopodite (which is severed with scissors) according to the method described by Aiken (1980).

Table 2.1. Molt cycle classification and associated pleopod stages in *Homarus*.

Molt stage[a]	Pleopod stage[b]	General characteristics[c]
C_3	0	Postmolt. Branchial carapace easily depressed by finger pressure, but rigid elsewhere.
C_4	0	Intermolt. All parts of carapace rigid.
D_0	1.0 to 2.5	Passive premolt. Epidermis retracts from cuticle.
D_1	3.0 to 4.0	Active premolt (irreversible). New epicuticle forms and new setae invaginate to maximum depth.
D_2	4.5 to 5.0	New exocuticle formed.
D_3	5,5	Extensive reabsorption of minerals from

[a] Modern version of system developed by Drach (1939). From Aiken (1973).

[b] From Aiken (1973).

[c] From Aiken (1980).

Premolt lobsters were identified as those with varying degrees of retraction of the epidermis and setal cones from the cuticle, represented by pleopod stages 1.0 to 5.5. In the absence of epidermal retraction (pleopod stage 0), shell rigidity was used to distinguish molt stages. Lobsters in which only the ventrolateral carapace could be depressed by light pressure were considered stage C3 (late postmolt) compared to those considered in stage C4 (intermolt), which were hardened all over (Aiken, 1980).

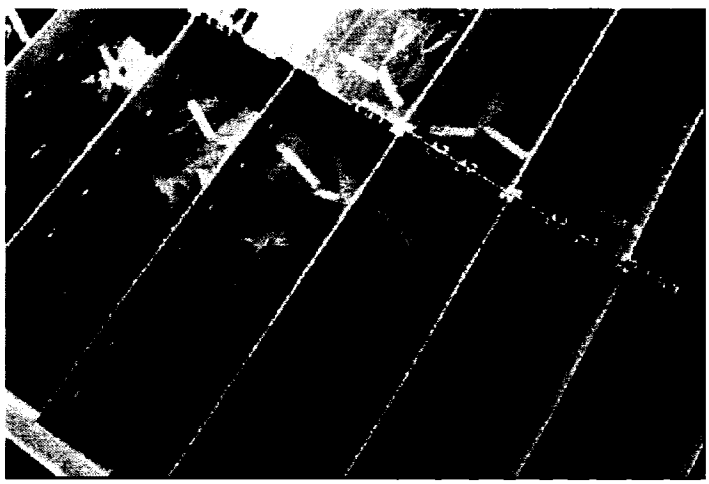
2.3.3 Lobster storage method

After the lobsters were sampled, they were placed in numbered slots in lobster pound storage trays (Figure 2.2 (a)). All trays were randomly allocated to a storage slot within the same storage rack (Figure 2.2 (b)). This storage rack was supplied with partly recirculated seawater at a rate of 106 to 121 l/min. System water quality parameters were monitored regularly (at least twice per week) for dissolved oxygen, ammonia, pH, salinity, and temperature. All parameters were maintained within acceptable limits for *Homarus americanus* (Van Olst et al. 1980).

2.3.4 Shell disease observations

Lobsters were observed monthly for shell disease lesions. Lesion characteristics were recorded, including width (mm), location (claw, body, tail), and number of lesions at each location. A shell disease rating system was used to score

(a)



(b)

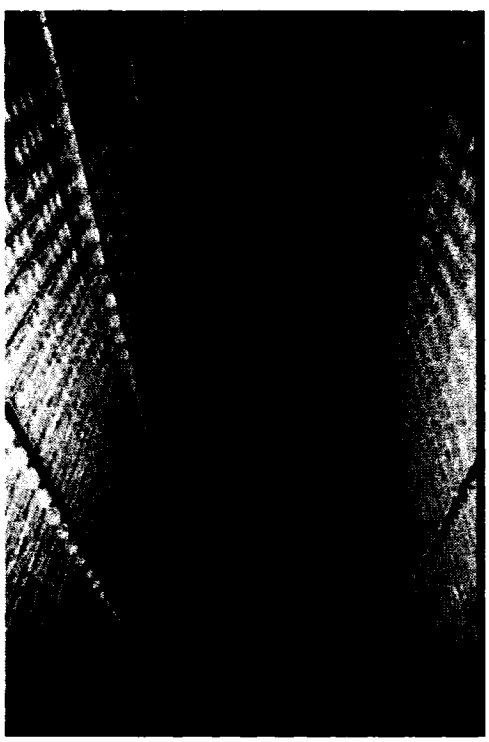


Figure 2.2. Lobster pound trays showing individual lobster compartments (a) and stacking system for long term storage (b) (Clearwater Seafoods photo).

infections based on both the frequency and size of the lesions (Table 2.2). Photos depicting lobsters at various shell disease scores are shown in Figure 2.3. For the inspection process, three trays of lobsters were removed simultaneously from the storage rack and each lobster was examined individually with the aid of a light, without removing or touching the lobsters. As a result, only lesions on the dorsal surface of the lobster were recorded.

Lesion characterization was based on the personal experience of the examiner and the normal sequence of events of shell disease, as described in published literature (Sindermann, 1989; Young and Pearce, 1975; Malloy, 1978). Initial bacterial invasion was identified by the presence of a white halo in the cuticle around the site of injury, followed by melanization of the area producing typical blackened lesions. All observations and measurements were performed by the same person.

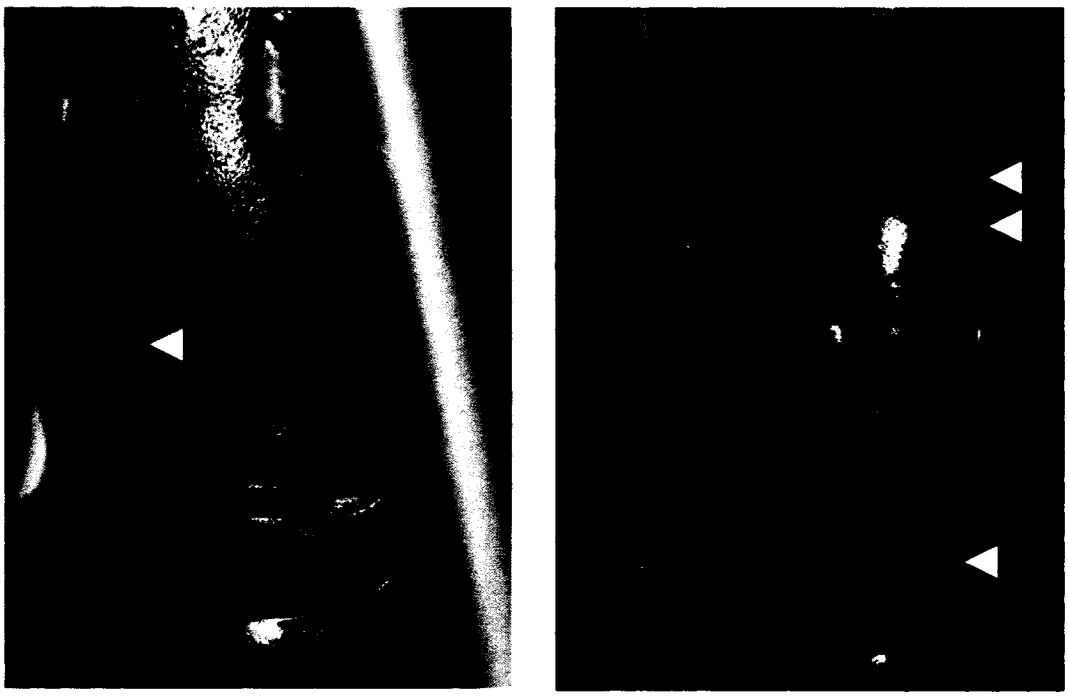
In total, four sample times were used (January, February, March, April) with successive samplings conducted in a blind manner, without knowledge of the animal's disease history. To control for the effect of sludge or organic accumulation, 252 lobsters on 7 randomly selected trays of lobsters were rinsed using a seawater hose prior to monthly disease observations. Mortalities were removed on a monthly basis.

Table 2.2. Rating system used to score shell disease infections.

Shell Disease Score	Frequency of Lesions	Size of Lesions
1	1	<1.0 cm
2	2-10	<1.0 cm
3	2-10	>1.0 cm
4	11-20	<1.0 cm
5	11-20	>1.0 cm
6	>20	

(a) Score 1

(b) Score 3



(c) Score 6

Figure 2.3. Photos depicting examples of lobsters identified as shell disease scores 1, 3, and 6.

2.3.4.1 Cumulative incidence

At each time point of the study, a new variable that indicated if a lobster ever had shell disease lesions at or prior to that time point was generated. Cumulative incidence of the group/subgroup was then determined; this was preferred over prevalence (presence of shell disease at each time point, regardless of whether they had shell disease previously) for the following reasons: we knew that all lobsters were free of disease at the start of the study, making cumulative incidence determination possible. Furthermore, cumulative incidence was deemed most applicable from an industry perspective. Within a long term storage facility, animals with any sign of shell disease are removed immediately, regardless of severity. These shell diseased lobsters are downgraded or identified as a poorer quality product destined for less lucrative markets such as immediate local sales or processing. Also, due to the apparent infectiousness of the disease (Fisher et al. 1976; Bullis, 1989; Sindermann, 1989) and poor ability for shipping and survival of infected animals, it is uncommon for even minor cases of the disease to be mixed in with uninfected lobsters. Also, an incidence rate (number of cases developing per unit of time of exposure) could not be accurately calculated because it was unknown as to when each lobster developed shell disease between monthly examinations.

2.3.4.2 Descriptive statistics

Descriptive statistics (proportions, means, and standard deviations) were calculated to determine average lesion characteristics, patterns in cumulative

incidence over time, and patterns in independent variables (by dealer, molt stage, and sex). Differences in raw values (among variables) for total protein, shell hardness, sex, and molt stage were evaluated using t-tests (continuous or ordinal variables) and chi-square tests (dichotomous variables). All statistical analyses were performed using Stata 6 (StataCorp, 1999).

2.3.4.3 Categorizing variables

Based on current industry practices, the raw values of continuous and ordinal variables were also converted into new dichotomous or ordinal variables to permit the results to assist decision-making (yes/no) with regard to whether a lobster or group of lobsters will be kept for long term storage if they have certain pre-storage test values. Total protein was categorized into low, medium, and high (less than 40 mg/ml, 40-60 mg/ml and >60 mg/ml, respectively). Shell hardness and molt stage were dichotomized into soft/medium and hard shelled (0-90 durometer units and >90 durometer units, respectively), and intermolt and premolt (pleopod stage 0 and pleopod stages 1.0 to 5.5, respectively). The transformed variables and their definitions are listed in Table 2.3. All statistical analyses were performed using Stata 6 (StataCorp, 1999).

Using these transformed variables, statistical analyses were conducted on factors affecting cumulative incidence of shell disease for each time point separately. Initial univariate analyses using relative risks were performed to identify those variables with at least a trend toward an unconditional association ($p<0.20$) with

Table 2.3. Description of data by variable and level.

Variable	Levels	Description
Total Protein	0,1,2	0 is TP > 60mg/ml; 1 is TP between 40-60 mg/ml; 2 is TP <40 mg/ml
Shell Hardness	0,1	0 is hard shell; 1 is medium/soft shell
Dealer	0,1	0 is primarily inshore; 1 is primarily midshore
Sex	0,1	0 is male; 1 is female
Molt Stage	0,1	0 is premolt; 1 is intermolt
Wash	0,1	0 is unwashed; 1 is washed
Disease	0,1	0 is no lesions; 1 is one or more lesions

shell disease cumulative incidence from the start of the study to the time point analyzed.

2.3.4.4 Logistic regression model building

Based on the cumulative incidence data and number of variables included in this study, a logistic regression model building approach (Hosmer and Lemeshow, 1989) was employed using disease (yes/no) as the dependent variable for each time period. The ordinal variable total protein was entered into the model using dummy variables.

The presence of confounding was assessed at each time period by initially offering all variables that met the initial cut-off criterion of $p<0.25$ to the model and utilizing a backwards elimination process to remove non-significant ($p>0.05$) variables. This process started with the full multivariate model, and then each of the six variables was examined individually on its significance, the least significant variable was removed, and then the change in the regression coefficients of the remaining variables was examined when it was removed from the model. Only non-significant variables whose removal induced an 'important' change (i.e. at least 25% for β's larger than 0.40 or smaller than -0.40, and at least 0.1 absolute change for β's between –0.40 and 0.40) in the estimates of the remaining coefficients were deemed to be confounders and remained in the model.

To assess for the presence of interaction in the final model, cross-product terms between the remaining variables were added to the model and their resulting

coefficients were examined for statistical significance using multiple Wald tests, with non-significant interaction terms being removed from the final model using a similar backward elimination process as described above (Hosmer and Lemeshow, 1989).

The significance of the final models was determined using likelihood ratio tests, comparing the likelihood of the 'full' model containing all significant predictors to that of the 'null' model containing only the intercept. Likelihood test statistics which are significant suggest that the variables do contribute significantly to the prediction of the outcome. The final models were also evaluated by determining their fit using the Pearson $\chi 2$ and the Hosmer-Lemeshow goodness-of-fit tests (Hosmer and Lemeshow, 1989). Each model's predictive ability was assessed by computing its sensitivity, specificity, and receiver operating characteristics (ROC). The ROC curve plots the sensitivity versus one minus the specificity. An ideal area under the curve is 1.0 while a random classifier would achieve an area under the curve of approximately 0.5.

Various diagnostic parameters including Pearson standardized residuals, leverage, and delta betas were calculated to identify poorly fit and highly influential observations. Odds-ratios were calculated from coefficients of final fitted models. All statistical analyses were performed using Stata 6 (StataCorp, 1999).

2.4 Results

2.4.1 Initial health assessment results

A summary of initial health assessment sample results (total protein, shell hardness, molt stage, and sex) of the study population, sorted by fishing area, is shown in Table 2.4. There were no significant differences in carapace lengths because only animals between 1.00 and 1.15 lbs. were selected for inclusion in this study, and therefore this variable was not included in the table.

No lobsters from either source were observed at molt stage greater than D_0 (i.e. no pleopod stages above 2.5 observed). All lobsters exhibited hardened ventrolateral carapaces, suggesting that no stage C_3 or postmolt individuals were included.

Significant differences in test results were observed between dealers, with lobsters from the primarily midshore dealer exhibiting lower total protein levels, softer shells, and a higher percentage of intermolt lobsters, compared to the primarily inshore dealer.

Irrespective of dealer, intermolt lobsters showed significantly lower total protein levels and softer shells. As molt stage increased from stage C4 (pleopod stage 0) to pleopod stage 2.5, total protein means also increased from 44.7 mg/ml to 90.6 mg/ml, respectively. The largest increase was observed between molt stage C4 (pleopod stage 0) and pleopod stage 1.0 lobsters, where mean total protein increased by 58% to 70.7 mg/ml. Total protein and molt stage were found to be moderately

Table 2.4. Initial total protein (mean ± standard deviation) and shell hardness (mean ± standard deviation) sample results by dealer, molt stage, and sex. Similar superscripts indicate non-significant differences ($p \leq 0.05$).

			n			TP		Shell hardness	
			male	female	total	(mg/ml)		(durometer units)	
Combined	**Molt Stage**	**0**	202	191	**393**	44.7 ± 18.2		87 ± 5.6	
		1,0	36	26	**62**	70.7 ± 17.7	a	91 ± 3.0	a
		1,5	30	13	**43**	73.1 ± 12.8	a	90 ± 2.7	a
		2,0	16	11	**27**	79.6 ± 16.8	a,b	91 ± 3.4	a
		2,5	10	5	**15**	90.6 ± 13.7	b	91 ± 2.1	a
	Sex	**Male**	294			54.0 ± 22.2	a	88 ± 5.3	a
		Female		246		51.7 ± 22.5	a	89 ± 5.2	a
		TOTAL	**294**	**246**	**540**	**53 ± 22.4**		**88 ± 5.2**	
Midshore	**Molt Stage**	**0**	122	109	**231**	38.3 ± 14.6		86 ± 5.8	
		1,0	13	14	**27**	63.6 ± 18.5	a	91 ± 3.5	a
		1,5	11	3	**14**	75.3 ± 11.8	a,b	90 ± 3.1	a
		2,0	7	2	**9**	66.6 ± 16.1	a,b	91 ± 3.0	a
		2,5	4	3	**7**	83.5 ± 12.7	b	91 ± 2.8	a
	Sex	**Male**	157		**157**	44.9 ± 20.0	a	86 ± 5.9	
		Female		131	**131**	43.9 ± 19.0	a	88 ± 5.3	
		TOTAL	**157**	**131**	**288**	**44.4 ± 19.6**		**87 ± 5.7**	
Inshore	**Molt Stage**	**0**	80	82	**162**	53.8 ± 18.9		88 ± 5.0	a
		1,0	23	12	**35**	76.1 ± 15.0	a,b	91 ± 2.5	a
		1,5	19	10	**29**	72.1 ± 13.3	a	91 ± 2.5	a
		2,0	9	9	**18**	86.1 ± 13.3	b,c	91 ± 3.6	a
		2,5	6	2	**8**	96.8 ± 12.0	c	92 ± 1.3	a
	Sex	**Male**	137		**137**	64.4 ± 20.0	a	89 ± 4.0	a
		Female		115	**115**	60.7 ± 22.8	a	89 ± 7.9	a
		TOTAL	**137**	**115**	**252**	**62.7 ± 21.4**		**89 ± 4.4**	

correlated (r=0.61, p=0.000), suggesting that in winter-harvested LFA 34 lobsters, total protein may be a useful indicator of molt stage.

Shell hardness was also significantly lower in intermolt lobsters, compared to premolt lobsters (mean durometer reading of 87 versus 91, respectively). However, unlike total protein, there was no difference in shell hardness among premolt lobsters (pleopod stages 1.0 to 2.5). Shell hardness and total protein show a moderate correlation (r=0.52, p=0.000).

There was no statistically significant difference in total protein or shell hardness by sex. There were significant differences in molt stage among the males and females with the females exhibiting a higher proportion of intermolt lobsters (77.6% compared to 68.7% in the males). Sex did not appear to be influenced by dealer with similar sex ratios (proportion males: females) observed for both the midshore dealer and the inshore dealer.

2.4.2 Shell disease descriptive results

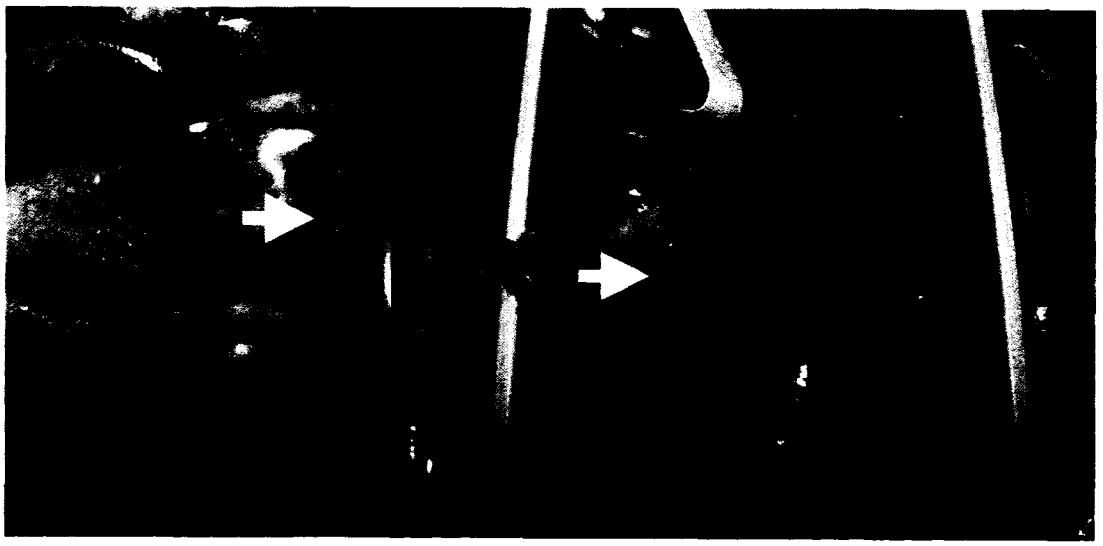
2.4.2.1 General characteristics of lesions

Observation of the pattern of lesion distribution (Table 2.5) showed a bilaterally symmetrical appearance in 70.1% of the infected lobsters and a unilateral appearance in 29.9% of the infected lobsters (Figure 2.4).

Table 2.5. Proportion of shell disease infections by lesion pattern and anatomical region.

		n	%
Lesion Pattern	**Bilateral**	197	70.1%
	Unilateral	84	29.9%
Exoskeletal Region	**Claw**	30	10.7%
	Carapace	76	27.0%
	Tail	88	31.3%
	> 1 Region	85	30.2%
	All Regions	28	10.0%

(a) Unilateral lesions



(b) Bilateral lesions

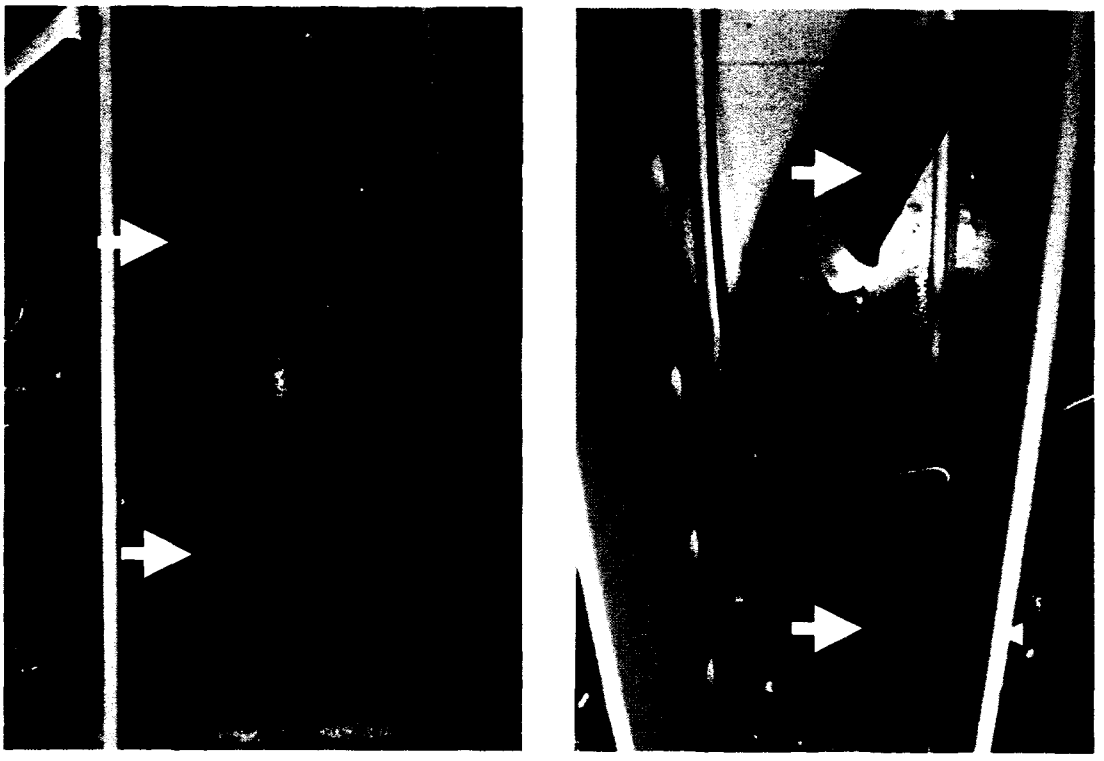


Figure 2.4. Photos depicting examples of lobsters with unilateral and bilateral shell disease lesions.

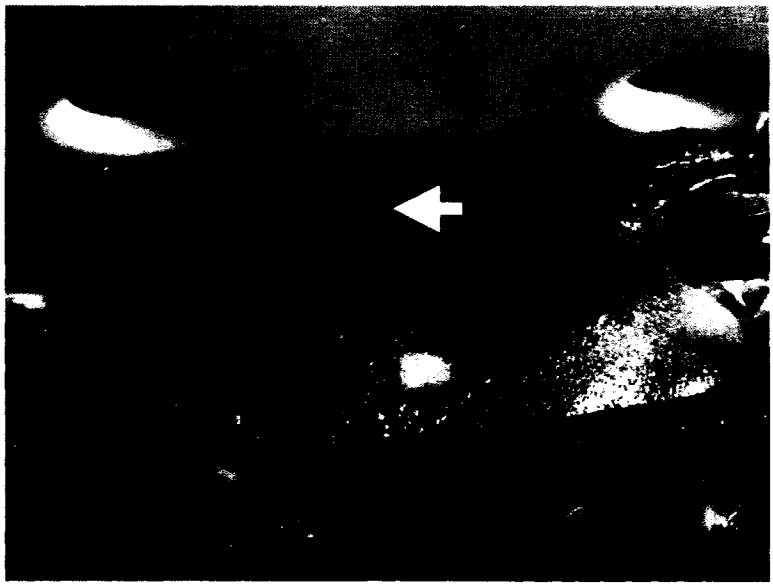
The bilateral lesions generally began as hyperpigmentation of the cuticular setae located over the surface of the body. This hyperpigmentation was indicated by the presence of small, round pits bounded by white or reddish margins (Figure 2.5 (a)). As the disease advanced, the resulting pigmented areas became enlarged, with adjacent pits eventually joining to form larger areas of erosion (Figure 2.5 (b)).

At 30 days, there was an average of 2.0 lesions per infected animal with the mean lesion size being 6.6 mm (Table 2.6). By 90 days, mean lesion size increased to 17.5 mm with an average of 11.3 lesions per animal. The observed reduction in mean frequency of lesions at 120 days was due to mortality of some of the worst affected lobsters. The proportion of lobsters at each shell disease score also indicated an increase in the severity of the disease. After the first 30 days, 54.0% and 0% of the infections were classed as score 1 (single lesion) and score 6 (more than 20 lesions), respectively, while at 90 days 23.4% and 24.4% of the infections were score 1 and 6, respectively.

2.4.2.2 Cumulative incidence

Cumulative incidences, by potential risk factors and in total, are shown in Table 2.7. Shell disease cumulative incidences observed in this study ranged from 25.7% at 30 days to 52.4% at the final time period of 120 days. The majority of new cases of shell disease were observed within the first 60 days of storage. After 60

(a)



(b)

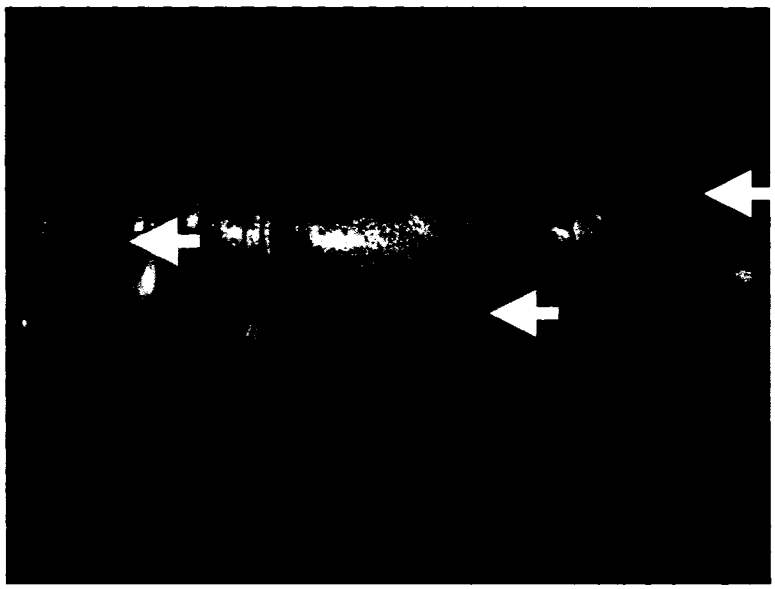


Figure 2.5. Photos depicting examples of lobster at various stages of shell disease development. Figure (a) shows the initial small, round pits while Figure (b) shows the enlarged areas of erosion.

Table 2.6. General characteristics of shell disease infections including mean lesion size (± standard deviation), mean lesion frequency (± standard deviation), and proportion of lobsters at each shell disease score. Superscripts denote statistical differences.

		30 days	60 days	90 days	120 days
Lesion Size (mm)		6.6 ± 5.8^{a}	13.3 ± 15.1^{b}	17.5 ± 20.7^{b}	18.1 ± 22.5^{b}
Lesion Frequency		2.0 ± 1.5^{a}	7.5 ± 8.2^{b}	11.3 ± 11.4^{c}	9.1 ± 9.1^{b}
Shell Disease Score	**1**	54.0%	25.9%	23.4%	28.1%
	2	35.3%	30.5%	21.3%	21.3%
	3	10.8%	24.1%	15.7%	14.0%
	4	0.0%	4.1%	6.6%	6.2%
	5	0.0%	5.5%	8.6%	11.8%
	6	0.0%	10.0%	24.4%	18.5%

Table 2.7. Cumulative incidence of shell disease (proportion of animals with observed lesions) at each sampling interval. Confidence intervals are shown in brackets.

		n	30 days		60 days		90 days		120 days	
Dealer	**Nearshore**	252	20.2%	(15.4 to 25.7%)	34.9%	(29.0 to 41.2%)	37.7%	(31.7 to 44.0%)	41.3%	(35.1 to 47.6%)
	Midshore	288	30.6%	(25.3 to 36.3%)	53.5%	(47.5 to 59.3%)	59.0%	(53.1 to 64.8%)	62.2%	(56.3 to 67.8%)
Molt	**Premolt**	147	8.2%	(4.3 to 13.8%)	18.4%	(12.5 to 25.6%)	19.0%	(13.0 to 26.3%)	23.1%	(16.6 to 30.8%)
	Intermolt	393	32.3%	(27.7 to 37.2%)	54.7%	(49.6 to 59.7%)	60.3%	(55.3 to 65.2%)	63.4%	(58.4 to 68.1%)
Shell	**Hard**	330	16.1%	(12.3 to 20.5%)	31.2%	(26.2 to 36.5%)	35.5%	(30.3 to 40.9%)	39.7%	(34.4 to 45.2%)
	Medium/Soft	210	41.0%	(34.2 to 47.9%)	66.2%	(59.4 to 72.6%)	70.5%	(63.8 to 76.6%)	72.4%	(65.8 to 78.3%)
Total	**<40**	209	46.9%	(40.0 to 53.9%)	79.4%	(73.3 to 84.7%)	84.2%	(78.5 to 88.9%)	86.6%	(81.2 to 90.9%)
Protein	**40-60**	131	16.0%	(10.2 to 23.5%)	29.8%	(21.1 to 38.4%)	38.2%	(29.8 to 47.1%)	43.5%	(34.9 to 52.4%)
(mg/ml)	**>60**	200	10.0%	(6.2 to 15.0%)	18.5%	(13.4 to 24.6%)	19.5%	(14.3 to 25.7%)	22.5%	(16.9 to 28.5%)
Wash	**Yes**	288	28.1%	(18.0 to 28.7%)	46.5%	(40.6 to 52.4%)	51.4%	(45.5 to 57.3%)	54.9%	(48.9 to 60.7%)
	No	252	23.0%	(23.0 to 33.7%)	42.9%	(36.7 to 49.2%)	46.4%	(40.1 to 52.8%)	49.6%	(43.3 to 56.0%)
Sex	**Male**	294	22.8%	(18.1 to 28.0%)	40.5%	(34.8 to 46.3%)	45.9%	(40.1 to 51.8%)	48.0%	(42.1 to 53.8%)
	Female	246	29.3%	(23.7 to 35.4%)	50.0%	(43.6 to 56.4%)	52.8%	(46.4 to 59.2%)	57.7%	(51.3 to 64.0%)
	TOTAL	**540**	**25.7%**	**(22.1 to 29.7%)**	**44.8%**	**(40.6 to 49.1%)**	**49.1%**	**(44.8 to 53.4%)**	**52.4%**	**(48.1 to 56.7%)**

days, relatively few animals developed shell disease. However, these cumulative incidence levels should be interpreted with caution because the increased handling that these study lobsters underwent during the study may have increased the incidence somewhat, particularly the washing, as discussed below.

By dealer, lobsters from the primarily midshore dealer showed higher cumulative incidence at each time, with 62.2% of lobsters infected by 120 days, compared to 41.3% for lobsters from the primarily inshore dealer. Significant differences were also seen by molt stage. Shell disease for intermolt lobsters (stage C4) ranged from 32.3% to 63.4%, while results for premolt lobsters (stage D0) ranged from 8.2% to 23.1% at 30 and 120 days, respectively.

Results by total protein and shell hardness also showed a similar trend of increased shell disease with decreased total protein and shell hardness. Lobsters with the lowest total protein levels (<40 mg/ml) exhibited 88.7% shell disease by 120 days storage, compared to only 23.5% in animals with the highest total protein levels (>80 mg/ml). Similarly, lobsters with the softest shells exhibited the highest cumulative incidence, with 85.7% shell disease at 120 days. Sludge removal and sex, did not appear to have much of an influence on shell disease cumulative incidence.

2.4.3 Shell disease logistic regression models

All risk factors were selected for inclusion in a predictive multivariate logistic regression model (Hosmer and Lemeshow, 1989). Using a backwards

elimination process, dealer and sex were found to be insignificant predictors in all four final models. At 30 days, total protein, shell hardness, and molt stage showed significant associations with shell disease while controlling for the presence of the other variables in the model. At 60 days, only total protein remained while at 90 days, total protein, molt stage and sludge removal remained as significant predictors of shell disease. At 120 days, total protein and sludge removal showed significant associations.

No variables were identified which met the criteria for confounding and subsequently none were forced into the models. None of the interaction terms tested was significant, suggesting that there was also no evidence of interaction in the four models. Results of the Pearson and Hosmer-Lemeshow tests were not statistically significant (Appendix A), indicating that there was no evidence that the data do not fit the models. The likelihood test statistics (Appendix A) for all four models were highly significant suggesting that the variables did contribute significantly to the prediction of the outcome. Evaluation of poorly fit observations showed no outliers in any of the models.

Using a cutpoint of 0.5, models at times 60, 90 and 120 days showed the best sensitivity and specificity (Appendix A), suggesting reasonable predictive abilities. At 30 days, the model's sensitivity, at 51.8%, was reduced, although the specificity was similar to that of other models. The area under the receiver operating curve (ROC) ranged from 0.750 at 30 days to 0.869 at 90 days, indicating moderate predictive ability for all models.

2.4.3.1 Logistic regression final models

The final models are shown below as coefficients.

30 days: ln [p/(1-p)] = -2.62 + 0.47Shell + 0.19Mid TP + 1.45Low TP + 0.73Molt +ϵ

60 days: ln [p/(1-p)] = -1.81 + 0.84Mid TP+ 3.08Low TP + ϵ

90 days: ln [p/(1-p)] = -3.24 + 1.03Mid TP + 3.49Low TP + 0.86Molt + 0.52Sludge +ϵ

120 days: ln [p/(1-p)] = -2.48 + 0.82Mid TP + 3.31Low TP + 0.66Sludge +ϵ

where ln [p/(1-p)] = the natural logarithm of the cumulative incidence of shell disease divided by 1 - the cumulative incidence of shell disease (the normal format of the dependent variable of logistic regression), coefficients for Mid TP (TP = 40-60 mg ml^{-1}) and Low TP (<40 mg ml^{-1}) indicate log odds relative to the High TP (TP >90 mg ml^{-1}); coefficient for Molt indicates log odds of intermolt lobsters relative to premolt lobsters; coefficient for Shell indicates log odds of soft/medium shelled lobsters ($\leq$90 durometer units) relative to the referent category of hard shelled lobsters (>90 durometer units); and coefficient for Rinse indicates log odds of sludge being rinsed off lobster backs relative to unclean lobsters.

Table 2.8 shows the final model results as odds ratios. Also included in this table are the odds ratios for the variables when they were not significant ($p<0.05$) at the time points. These odds ratios were calculated after each non-significant variable was added individually to each of the final models at each time point, to demonstrate how close (or not) each of these variables were to being significant at that time point.

Table 2.8. Odds ratios, standard errors, p values and 95% confidence intervals for the final logistic regression models at 30, 60, 90, and 120 days.

30 days

Predictor	Odds Ratio	S.E.	p	95% C.I.
Mid total protein'	1.21	0.44	0.598	0.59-2.45
Low total protein"	4.24	1.45	0.000	2.17-8.30
Intermolt stage	2.07	0.78	0.053	0.99-4.33
Medium/soft shell	1.61	0.39	0.051	1.00-2.59
Washed	1.47	0.32	0.076	0.96-2.26

60 days

Predictor	Odds Ratio	S.E.	p	95% C.I.
Mid total protein'	2.33	0.66	0.003	1.34-4.05
Low total protein"	21.77	5.73	0.000	12.99-36.48
Intermolt stage	1.61	0.50	0.124	0.88-2.97
Medium/soft shell	1.16	0.29	0.540	0.71-1.90
Washed	1.36	0.30	0.161	0.88-2.08

90 days

Predictor	Odds Ratio	S.E.	p	95% C.I.
Mid total protein'	2.83	1.02	0.004	1.40-5.74
Low total protein"	32.76	11.66	0.000	16.31-65.81
Intermolt stage	2.36	0.90	0.025	1.12-5.00
Medium/soft shell	1.47	0.38	0.142	0.88-2.45
Washed	1.68	0.41	0.033	1.04-2.70

120 days

Predictor	Odds Ratio	S.E.	p	95% C.I.
Mid total protein'	2.27	0.67	0.005	1.28-4.04
Low total protein"	27.58	7.67	0.000	15.99-47.56
Intermolt stage	1.54	0.50	0.181	0.82-2.89
Medium/soft shell	1.07	0.28	0.800	0.64-1.78
Washed	1.95	0.45	0.004	1.24-3.06

' Hemolymph total protein levels between 40 and 60 mg/ml
" Hemolymph total protein levels less than 40 mg/ml

After 30 days storage, having low total protein (<40 mg/ml) increased the odds of developing shell disease by 4.24 times compared to high total protein lobsters. Molt stage and shell hardness also appeared as moderate predictors with the odds of developing shell disease 2.07 and 1.61 times greater in intermolt staged and soft/medium shelled animals, respectively.

At 60 days, total protein remained as the only significant predictor with the odds of developing shell disease being 21.77 times greater in animals with low total protein compared to those with high total protein.

At 90 days, total protein again emerged as the strongest predictor with the odds of developing shell disease being 32.76 times greater in low total protein lobsters, and 2.83 times greater in mid protein lobsters compared to high total protein lobsters. Molt stage was also significant at this time period with the odds of shell disease being 2.36 times greater in intermolt animals compared to premolt animals. Sludge also appeared as a moderate predictor at this time with the odds of developing shell disease being 1.68 times greater in animals which were washed to remove the sludge compared to those on which the sludge was left intact.

At the final time period of 120 days, total protein and sludge remained as the only significant predictors. The odds of shell disease were 2.27 times greater in mid protein lobsters and 27.58 times greater in low protein lobsters compared to high total protein lobsters, and 1.95 times greater in lobsters with sludge removal.

At all time periods, low total protein emerged as the strongest predictor of shell disease. Despite a moderate correlation with total protein (r=0.61), molt stage remained as a significant predictor at 30 and 90 days. Having a soft/medium shell,

compared to a hard shell, appeared to contribute to the development of shell disease only at 30 days. In all later models, shell hardness was no longer a significant predictor. Sludge removal, in comparison, emerged as a significant predictor in the two final models at 90 and 120 days, with the odds of developing shell disease moderately greater in washed lobsters compared to the unwashed lobsters.

2.5 Discussion

Shell disease development in captive populations has typically been associated with poor husbandry (Sindermann, 1989; Getchell, 1991) resulting in exoskeletal damage. In our study, however, we saw that shell disease can occur with high cumulative incidences in the absence of obvious physical damage to the shell (all animals were initially free of lesions and no physical damage was possible in the storage facility), suggesting that mechanical trauma is not a necessary prerequisite for lesion development. This supports the belief that, in an otherwise healthy or injury-free animal, other factors, such as poor physiological or nutritional condition, may increase a lobster's susceptibility to developing shell disease (Bullis, 1989).

Our analysis of the potential risk factors for shell disease in a lobster pound facility suggested that the inherent physiological differences in groups of lobsters lead to shell disease, with total protein as the main indicator of this difference. In particular, results of logistic regression analysis showed that the odds of developing shell disease were as much as 33 times higher for lobsters with low total protein levels versus lobsters with high protein levels.

A number of criteria need to be fulfilled to definitively state that low protein is actually a risk factor for shell disease within an impoundment facility. Temporality (the purported cause is before the disease occurrence) is achieved in this analysis, because the low protein levels were detected at the start of the storage period in lobsters that did not have shell disease and subsequently developed the disease. Our analyses also discovered a statistically significant dose:response relationship whereby lobsters with lower protein levels had very high odds of developing the disease, while lobsters with moderate protein levels had moderately high odds of developing the disease. There is also biological plausibility to this discovered association. Low total protein levels lead to inadequacies in the lobster's protein-requiring processes of cuticular maintenance, wound repair, and/or internal defense mechanisms. Improper nutrition has also previously been associated with exoskeletal abnormalities, specifically epicuticular deformities (Gallager et al. 1978), and excessive melanization (Lightner et al. 1979). While the association between reduced total protein and poor physiological condition has been established, previous efforts to develop predictive models based on this factor have been unsuccessful (Prince, 1997).

Another criterion for causality is that the study does not have known significant bias, either selection, misclassification or confounding bias. Our study utilized a random selection process of normal sized lobsters from an area known to have shell disease in the past. Therefore, significant selection bias is unlikely. With regard to misclassification bias, the protein levels were determined using a refractometer with known reliability for taking protein levels in hemolymph (Leavitt

and Bayer, 1977; Ozbay and Riley, 2002), and recalibrated regularly. Classifications into low, moderate and high categories of protein were conducted based on current industry practices. Also, significant misclassification bias in the disease status is unlikely since the disease evaluations were performed in a blind manner by the same examiner.

With regard to confounding bias, data on other possible confounding variables were collected and offered to the final models. However, even while controlling for some of these other variables, there was still a significant association between protein levels and shell disease cumulative incidence.

The lobsters' molt stage emerged as an important predictor for shell disease in this study in the 30 and 90 day models, with a trend towards a relationship in the 60 and 120 day models as well. Because post-molt lobsters are more susceptible to shell disease, the timing of the fishery in Southwest Nova Scotia (SWNova) in relation to the molt cycle may explain the variability in shell disease seen in impoundment facilities year to year. The SWNova fishery is dominated by fall landings with an average of 48% of the catch occurring in the first 4 weeks of the season (DFO, 2001). While landings are higher, quality of this catch is generally poorer than that landed in the spring. The poorer quality observed in the fall has been attributed to the shorter recovery time between molt and the start of the fishery, as well as variable environmental conditions during this season which affect the lobster's nutritional status (Waddy et al. 1995).

Dealer did not emerge as a significant predictor of shell disease in the final models. However, initial sample results did show a significant difference in total

protein between the two dealers. Specifically, lobsters from the dealer who obtained lobster primarily from midshore grounds, which are known to be of poorer quality as compared to lobsters fished from the more traditional nearshore, had reduced total protein and shell hardness levels compared to the primarily nearshore lobster from the other dealer. Therefore, molt stage, total protein and hardness are intervening variables (Dohoo et al. 2003) for the dealer-shell disease relationship (see Figure 2.6); the variation in shell disease related to dealers is accounted for by these measured variables in the final models, preventing dealer from remaining in the final models. These results are significant because fishing effort continues to increase in this midshore area of Nova Scotia. By the late 1990's, it was reported to comprise approximately 20 to 30% of the LFA's total landings in Atlantic Canada (DFO, 2001).

Results of our study showed that sex was not a significant predictor for shell disease. This is contrary to previous studies of natural populations where females, especially ovigerous females, have generally demonstrated higher disease prevalence (Ziskowski et al. 1996; Young, 1991; Baross et al. 1978; Estrella, 1991). Mature females molt with only one-half the frequency of males (Aiken, 1980) and thus, potentially retain diseased exoskeletons for longer periods.

Shell hardness was only a moderate predictor in the 30 day model (odds of developing shell disease was 1.6 times greater in soft shelled than hard shelled

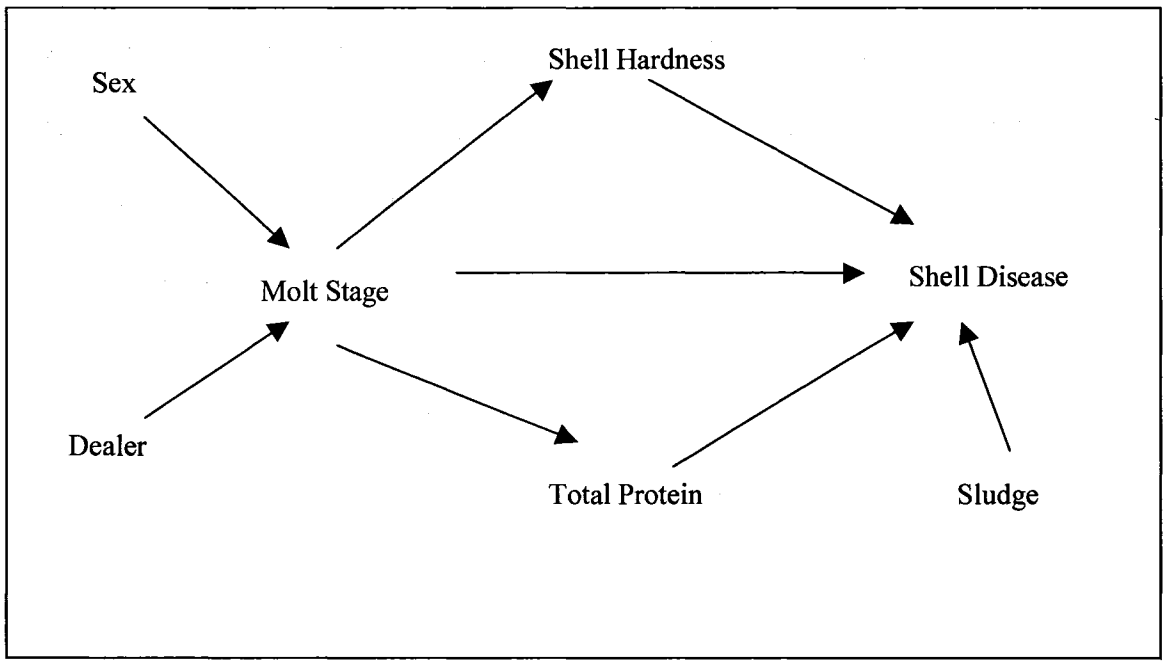


Figure 2.6. A causal diagram of factors affecting shell disease in impounded lobsters.

lobsters). There was a trend towards a relationship in the 90 day model, nevertheless, in the remaining final models, shell hardness was not a significant predictor and therefore is unlikely to be useful for predicting shell disease if molt stage and total protein are measured.

The effect of washing the lobsters showed unexpected results in the logistic regression models. In the models at 90 days and 120 days, the washed lobsters were more likely to develop shell disease than those for which the sludge was allowed to accumulate, suggesting that the sludge offers some protective benefits to the captive lobsters. It is unclear whether the increased susceptibility to shell disease was actually due to the absence of the sludge or simply a side effect of increased stress from the washing process. Without specific data on sludge levels, characteristics, and composition, a discussion of criteria for causality would be premature, but would be an interesting area for future research.

With a large number of criteria for causality fulfilled, there is strong evidence that low protein is a risk factor for shell disease. The only remaining criterion is consistency, whereby these results should be repeatable among other lobster populations by other independent researchers.

One question that remains to be answered is why some lobsters had low protein upon entry into the storage facility. Previous work has shown that the concentration of lobster hemolymph proteins varied directly with the lobster's nutritional condition as well as its amount of muscle (Stewart et al. 1967; Stewart et al. 1972). The cyclical changes in total protein with respect to the molt stage shown in this study support previous research. Stewart and Li (1969), Barlow and Ridgway

(1969), and Mercado-Allen (1991) also reported low total protein levels in post-molt animals with a gradually increase to high levels before ecdysis. These variations reflect physiological modifications such as re-absorption of exoskeletal minerals during pre-molt (Passano, 1960) and water uptake to increase body size immediately post-molt (Barlow and Ridgeway, 1969). This question does not need to be answered for total protein to be effectively used as an indicator of healthiness and predictor of the likelihood for developing shell disease during storage, particularly after other researchers have corroborated our findings.

Another remaining question for future research is how total protein could be used to determine whether or not to keep a shipment of lobster for long term storage. Simulation models could be run to determine the economic break-points in total protein, above which the losses due to shell disease are too high, at the load level. The reliability of these models could then be determined through data collection and analysis.

In conclusion, total protein has emerged as the strongest predictor of shell disease in winter caught lobsters from Southwest Nova Scotia, regardless of the length of time stored. However, our study also showed that other physiological and environmental factors are significant in the development of the disease. Molt stage remained a significant predictor in 2 of the models, with a trend towards significance in the remaining models, suggesting that other factors related to the molt cycle offer protection against shell disease. Shell hardness showed significance only in the 30 day model, suggesting that this factor may only be important early in the storage period. In a long term lobster pound storage facility, where the number of days held

generally exceeds 30 days, shell hardness may not be a useful indication of quality. Sludge accumulation was significant in 2 of the final models, with a trend towards significance in the remaining two models. These results suggest the sludge has a cumulative effect over time, with the greatest protective benefit seen in the 120 day model.

From the perspective of a long term storage facility, the quality of the lobsters entering the facility is critical and, as this study shows, determines the extent to which shell disease will develop. Unfortunately, the factors which influence the quality of the catch (such as timing of the molt, continued expansion into the midshore grounds, and an industry focus on quantity versus quality) are difficult to control or predict. Therefore, shell disease will continue to be a concern for impounded populations of the American lobster, and testing the quality of incoming lobsters to long term storage facilities (particularly total protein and molt stage) will continue to be of utmost importance.

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CHAPTER 3

GENERAL DISCUSSION

3 GENERAL DISCUSSION

The goal for this thesis was to identify potential risk factors for development of shell disease within a long term lobster impoundment facility. The results generated herein show that research data from impoundment facilities are a valuable resource in the understanding of factors affecting impoundment shell disease. In addition, by disclosing this information, this thesis hopes to help overcome some of the communication issues that continue to challenge the lobster industry.

The results of the epidemiologic study conducted within the lobster storage facility exhibited the importance of the physiological condition of the host in relation to susceptibility to shell disease. Total protein emerged as the strongest predictor of shell disease, with the odds of developing shell disease being as much as 33 times greater for lobsters with low total protein levels compared to lobsters with high protein levels. Molt stage also emerged as an important predictor, with post-molt animals being more susceptible. This association may explain the appearance of the disease in the fall Southwest Nova Scotia fishery, which are comprised primarily of post-molt animals.

The impact of environmental factors, such as seawater temperature, on the lobster's molt cycle may account for the annual variability in shell disease prevalence. Seawater temperature influences the rate of food consumption (Bordner and Conklin, 1981), and the timing of the molt cycle (Stewart and Squires, 1968), where cooler than normal water temperatures may result in a delayed molt and poor nutritional condition at the time of harvest. Examining the association between environmental conditions on the lobster's physiological and subsequent

susceptibility to shell disease during impoundment would be an interesting area for future research.

While there was an association between low protein levels and shell disease in winter pounded lobsters, the absence of shell disease in spring caught lobsters from LFA 34 is still unexplained. Interestingly, while shell disease is a re-occurring problem in the fall and winter, low protein lobsters caught and pounded from LFA 34 during April and May do not develop shell disease (Theriault, unpublished observations). Examining the physiological and environmental differences between the fall and spring lobsters from LFA 34 would also be an interesting area for future research. The effect of seawater temperature on the lobster's metabolism and immune system is believed to play an important role in the development of shell disease (Stewart, 1980). While post-harvest or holding temperatures have been implicated in shell disease development (Malloy, 1978), pre-harvest temperatures may also be significant. Lobsters harvested from the cold waters in the fall may lack ability to maintain shell condition or fight off bacterial invasion when placed in the impoundment situation.

Shell disease development in impounded Southwest Nova Scotia (LFA 34) lobsters has previously been reported (Hess, 1937; Malloy, 1978; Prince, 1997). Results of this study, however, show that even within this geographical area there are variations in susceptibility to shell disease. In this study, lobsters obtained from the primarily midshore dealer exhibited poorer initial physiological quality compared to the lobsters from the dealer who obtained his lobsters from the more traditional inshore areas.

While research into differences between nearshore and midshore lobsters is limited, it is known that the midshore area produces a higher percentage of mature animals (DFO, 2001). In addition, it was noted in this study that these lobsters also appear to have physical differences compared to nearshore lobsters, with more individuals exhibiting reddish coloration as opposed to the traditional dark green coloration of Southwest Nova lobsters. Lobsters of this reddish coloration were also anecdotally identified by Prince (1997) to be more susceptible to shell disease within the industry. More work should be done to further investigate regional differences in quality within LFA 34 since continued expansion into this relatively new midshore fishing area has the potential to dramatically impact on the industry through increased mortality and disease.

The prevalence of shell disease in this epidemiologic study (Table 2.7) was significantly higher than that reported from the production data for the same season (Theriault, unpublished observations). The lobster pound production data results showed a mean shell disease prevalence of 1.3% for lobsters from LFA 34 during the 2002 winter pounding season. This epidemiological study, in comparison, showed an overall cumulative shell disease prevalence of 25.7% for 30 days storage and 44.8% for 60 days storage. One possible explanation for this discrepancy is the fact that many of the minor shell disease lesions go unnoticed during the lobster pound production process. While the epidemiologic study recorded all observable lesions, production records likely do not include any of the less severe lesions (i.e. those less than 1cm in size). Based on this epidemiologic study (Table 2.6), as many as 90% of observed lesions at 30 days were less than 1 cm in size, and therefore,

difficult to detect within the production process. This suggests that the level of shell disease reported in the industry may actually be lower than the actual prevalence and that shell disease infections begin earlier than previously thought (in this case, after less than 1 month storage).

Management decisions pertaining to the length of time specific shipments spend in storage may also explain this discrepancy. Because the prevalence of shell disease increases with the number of days in storage (Table 2.7), during the winter pounding seasons, inventory levels are managed in order to minimize the number of days held (take out oldest product first). In addition, lobsters from traditionally poorer performing areas (such as dealer 1) are also targeted to minimize storage time.

Another possible explanation for the discrepancy is the additional handling incurred by the lobsters in the epidemiologic study. While not handled individually during the monthly sampling, the water supply was shut off and the trays themselves removed from the storage system until lesion observations were completed.

Overall, this study demonstrated the importance of the quality of the lobsters pounded on the success of a long term storage facility. Unfortunately, the factors which influence the quality of the catch (such as environmental conditions, timing of the molt, fishing area, and handling) are difficult to control or predict. Initiatives, however, are underway in LFA 34 to document and better understand the relationship between the timing of the lobster's molt cycle, environmental factors and subsequent lobster quality during harvest (Claytor, 2005). This project, while designed to monitor lobster quality (molt stage and total hemolymph protein), could

also help identify lobsters which would be more susceptible to developing shell disease during post-harvest long term storage.

While the quality of the lobsters being harvested is beyond the control of pound keepers, results of this study show there are still some options available for controlling, albeit not eliminating, shell disease. Increased attention on quality of the lobsters being selected for long term storage is essential. Each facility should have a monitoring program consisting of careful visual inspection to remove any weakened, damaged, or culled lobsters. Documentation of results on a individual shipment basis is also beneficial in order to track results and identify potential problem sources. Detailed inventory management, involving tracking shipments of lobsters during storage, while beneficial, may not be possible within most long term storage facilities.

In addition to a careful visual inspection, monitoring of pre-storage lobster biological parameters is also important. In terms of shell disease, total protein and molt stage appear to be the most significant predictors in lobsters from LFA 34. Molt staging, however, is likely not practical for most production facilities because it requires both laboratory facilities and specialized training. Total protein, however, is a more user friendly procedure, with information and training courses available (Lavallée, 2004). Implementation of a routine sampling procedure is recommended in order to help identify shipments which are more at risk for developing shell disease. In the absence of total protein and molt staging, shell hardness may be used as a general indicator of quality that, when combined with efforts to minimize the number of days in storage, may help reduce the impact of the disease.

Because the LFA 34 lobster season targets lobsters which are harvested after molting, the lobsters caught later in the season (with more time to recover from the molt) will be of better quality than those caught during the first few weeks of the season. Limiting the amount of lobsters purchased during the first few weeks, or at least minimizing the amount of time of storage for these early catches, will be beneficial.

Efforts should be made, when possible, to separate shipments by geographical source. Lobsters from boats which fish in the midshore areas are more likely to be of poorer quality compared to lobsters from the nearshore areas due to later onset of molt in the midshore area (Claytor, 2005).

In conclusion, while shell disease will continue to be a re-occurring concern for impounded populations of the American lobster, careful inspection, biological testing, and improved inventory management of pounded lobsters can help maintain manageable levels of shell disease within a long term storage facility.

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4 APPENDIX A

Results of Pearson and Hosmer-Lemeshow goodness-of-fit tests (chi square and p values) and predictive ability (sensitivity, specificity, and receiver operating characteristic) for logistic regression models.

	30 days		**60 days**		**90 days**		**120 days**	
Test	**chi-sq**	**P**	**chi-sq**	**P**	**chi-sq**	**P**	**chi-sq**	**P**
Pearson	6.93	*0.436*	0	-	6.71	*0.460*	1.27	*0.531*
Hosmer-Lemeshow	3.38	*0.641*	0	*1.000*	5.27	*0.380*	1.27	*0.860*
Sensitivity	51.8%		71.8%		78.2%		73.7%	
Specificity	81.5%		85.3%		86.6%		86.1%	
ROC	0.750		0.810		0.869		0.837	

Likelihood ratio statistics for logistic regression models

	30 days	**60 days**	**90 days**	**120 days**
Log-likelihood full model	-263,00	-268,17	-227,41	-254,43
Log-likelihood null model	-307,98	-367,42	-361,30	-366,42
Likelihood ratio statistic	89,95	198,51	267,78	223,99
P	0,000	0,000	0,000	0,000