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

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ABSTRACT A logistic regression model building approach was used to evaluate the association between the development of impoundment shell disease in an American lobster (*Homarus americanus*) storage facility, and prestorage physiological parameters (hemolymph total protein concentration, molt stage, sex, and shell hardness) and environmental factors (surface sludge accumulation). A total of 540 disease-free, winter-harvested lobsters from southwest Nova Scotia were tested prior to storage and then examined for signs of shell disease at 30, 60, 90, and 120 days of storage. Total protein concentration was the strongest predictor of shell disease, with the odds of developing shell disease ranging from 4.3–26.7 times higher for lobsters with low total protein concentration *versus* lobsters with high protein concentration, depending on the time point examined. Lobsters in the intermolt stage and removal of sludge from the surface of the lobster also emerged as important risk factors for shell disease development. These results reinforce the observation that the quality of lobsters entering an impoundment facility is critical and ultimately predicts the extent to which shell disease will develop.

KEY WORDS: lobster, *Homarus americanus*, shell disease, risk factors, impoundment

INTRODUCTION

Shell disease was first identified in impounded American lobsters (*Homarus americanus*, H. Milne Edwards, 1837) in 1937 (Hess 1937). There are three types of shell disease recognized in American lobsters: impoundment, burn (or rust) spot, and epizootic shell disease. Impoundment shell disease has recently been identified, based on pathological, etiological, and epidemiological characteristics, as being different from burn spot and epizootic lobster shell disease observed in wild populations of the American lobster (Smolowitz et al. 2002). Impoundment shell disease typically begins as a series of small, bilaterally symmetrical lesions, centered around setal pores on the dorsum of the animal (Bullis 1989). As the disease progresses, these pits commonly extend laterally such that adjacent pits join to form larger areas of erosion. These areas of erosion appear, histologically, as areas of cleanly removed cuticle with little or no cuticular matrix remaining (Smolowitz et al. 1992) resulting in the disease's characteristic "scooped out" appearance. In addition to this aesthetically unappealing appearance, diseased lobsters also exhibit elevated mortality during shipping and long term storage.

There seems to be a general consensus 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 might be a consequence of physical damage to the shell, or environmental or physiological stressors acting as immunosuppressants (Bullis et al. 1988). Several factors have been implicated as potential immunosuppressors contributing to shell disease development in healthy crustaceans, including environmental quality (Malloy 1978, Vogan & Rowley 2002), 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). Many of these potential risk factors, however, were identified from studies of shell disease in wild populations and, therefore, might not be applicable to impounded populations.

This study was designed to examine various environmental and physiological parameters as potential risk factors for shell disease development within a lobster impoundment facility. Specifically, the objective of this study is to use statistical models to determine associations, at the individual lobster level, between the development of shell disease within impounded populations of the American lobster, and the following potential risk factors: hemolymph total protein concentration ([TP]), shell hardness, molt stage, sex, dealer, and sludge accumulation.

MATERIALS AND METHODS

Experimental Animals

Lobsters used in this study were selected from a lobster storage facility in Arichat, Nova Scotia, Canada, between December 4 and December 14, 2001. Fresh caught lobsters were obtained from two commercial lobster dealers located in Lobster Fishing Area (LFA) 34, in southwestern Nova Scotia. Although the dealers were located in close proximity to one another, there were differences in their respective fishing grounds. The fleet from one dealer covered more of the midshore grounds (inside the offshore LFA 41 line west of Browns Bank in depths greater than 50 m) whereas the fleet from the other dealer stayed primarily in the traditional inshore fishing areas of LFA 34 (in depths <50 m).

A study population of disease-free, strong and undamaged lobsters, weighing between 454 g and 521 g, was systematically randomly chosen at regularly spaced time intervals during their size grading process. Sample sizes of 288 lobsters from dealer 1 (primarily midshore), and 252 lobsters from dealer 2 (primarily inshore) were obtained.

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Experimental Design

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 temperature compensated Atago refractometer for direct reading of the refractive index, and [TP] was calculated based on the modified biuret method (Layne 1957) using bovine albumin as the standard. Shell hardness, or carapace rigidity, was measured along the ventrolateral region of the carapace using a durometer (Hicks & Johnson 1987, Foyle et al. 1989). Sex determinations were performed and molt stage was determined using the setal staging method (Aiken 1980).

Immediately after sampling, the lobsters were placed sequentially in individual compartments in plastic storage trays. Each tray (123 cm long, 71.6 cm wide, and 12.5 cm high) housed a total of 36 lobsters (density of 41 lobsters per m^2). The filled trays were randomly allocated to a position within the same vertical storage racking system. This storage rack was supplied with partly recirculated seawater at a rate of 106–121 L min^{-1}. Water temperatures ranged between 1.5°C and 1.7°C and the lobsters were not fed, as is standard practice during long-term storage. System water quality parameters (ammonia, pH, and dissolved oxygen) were monitored daily and all parameters were maintained within acceptable limits for *Homarus americanus* (Van Olst et al. 1980).

Lobsters were observed at four monthly time periods (30, 60, 90, and 120 days of storage), and shell disease lesions were identified based on the normal sequence of events of shell disease development, as described in published literature (Sindermann 1989, Young & Pearce 1975, Malloy 1978). Initial bacterial invasion was identified by the presence of a white halo in the cuticle around the site of invasion, followed by melanization of the area, producing typical blackened lesions. To assess the role of surface sludge accumulation on the incidence of shell disease, a total of 4 trays (144 lobsters) from each of the two commercial dealers were randomly selected and all lobsters within these trays were rinsed monthly using a seawater hose.

Statistical Analysis

Correlations among all of the prestorage lobster biological parameters were determined using Pearson correlation coefficients. Descriptive statistics (means and standard deviations) were calculated for the hemolymph parameters, in total and by fishing area, molt stage and sex. Significant differences ($P < 0.05$) in [TP] and shell hardness between fishing areas, sexes, and molt stages were determined using *t*-tests. Cumulative incidence of shell disease (% of lobsters developing shell disease lesions to that time point) overall, and within each group/subgroup, was calculated.

The continuous and ordinal variables were converted into new ordinal or dichotomous variables based on industry standards. Hemolymph [TP] was classified into low (<40 mg mL^{-1}), medium (40–60 mg mL^{-1}), and high (> 60 mg mL^{-1}) categories. Shell hardness was dichotomized into soft/medium (0–90 durometer units) and hard (>90 durometer units). Molt stage was dichotomized into intermolt and premolt stages, referring to pleopod stage 0 and pleopod stages 1.0–5.5, respectively (Aiken 1973).

Logistic regression was used to determine univariate associations between incidence of shell disease and these transformed variables and the remaining dichotomous variables (sex, dealer, and sludge removal), to each time point, for each time point separately. A random effects term for storage tray was also included in the analysis to adjust for clustering. A backward elimination, logistic regression, model-building approach (Hosmer & Lemeshow 1989) was used to determine the factors associated with developing shell disease prior to each time point, for each time point separately, whereas controlling for confounding by other factors in the model. Variables remained in the model if they maintained a P value < 0.05 or demonstrated significant confounding.

The presence of confounding was assessed at each time point by initially offering all variables that met the initial cut-off criterion of $P < 0.25$ to the model and utilizing a backward elimination process to remove nonsignificant ($P > 0.05$) 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. Only nonsignificant 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 ($P < 0.05$) using multiple Wald tests, with nonsignificant interaction terms being removed from the final model using a similar backward elimination process as described above (Hosmer & Lemeshow 1989).

The significance of the final models was evaluated 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-square and the Hosmer-Lemeshow goodness-of-fit tests. These tests are based on the premise that the data will be divided into subsets and within each subset, the predicted number of outcome events will be computed and this will be compared with the observed number of outcome events (Dohoo et al. 2003). Results of these tests indicate whether there is sufficient evidence that the observed data do not fit the model. The Pearson chi-square test is based on dividing up the data into the natural covariate patterns, whereas the Hosmer-Lemeshow test is based on a more arbitrary division of the data. 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 variables in the final models. All statistical analyses were performed using Stata 8 (StataCorp 1999).

RESULTS

No lobsters were observed at setal stages greater than 2.5, and all lobsters exhibited hardened ventrolateral carapaces, suggesting that no postmolt individuals were included in the

study (Aiken 1980). Shell hardness showed moderate correlation ($r = 0.52$, $P < 0.001$) with [TP]. Total protein concentration and molt stage were also found to be moderately correlated ($r = 0.61$, $P < 0.001$).

Descriptive statistics of prestorage [TP] and shell hardness, sorted by fishing area, molt stage, and sex, are shown in Table 1. Intermolt lobsters showed significantly lower [TP] and softer shells compared with premolt lobsters. Lobsters from the primarily midshore dealer exhibited significantly lower [TP] compared with the primarily inshore dealer ($P < 0.05$). Midshore lobsters had a significantly higher proportion ($P < 0.001$) of intermolt lobsters compared with inshore lobsters (80.2% compared with 64.3%, respectively). Differences in molt stage were also observed by sex with a significantly higher proportion ($P = 0.020$) of the females in intermolt stage compared with the males (77.6% and 68.7%, respectively).

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 (Fig. 1). The majority of new cases of shell disease were observed within the first 60 days of storage. From the univariate analyses, lobsters from the primarily midshore dealer showed significantly higher cumulative incidence at each time period, as compared with lobsters from the primarily inshore dealer. Shell disease cumulative incidence for intermolt lobsters was significantly higher than for premolt lobsters at all of the time periods. At all time periods, lobsters with the lowest levels of [TP] (<40 mg mL^{-1}) exhibited higher cumulative incidence than lobsters with medium or high levels of [TP], whereas lobsters with medium/soft shells exhibited higher cumulative incidence than lobsters with hard shells. Female lobsters had a higher cumulative incidence of shell disease than male lobsters at 60 and 120 days. There was no significant difference in shell disease incidence with rinsing.

All risk factors were selected for inclusion in a predictive multivariate logistic regression model (Hosmer & Lemeshow 1989). Using a backward elimination modeling process, the dealer and sex variables were found to be nonsignificant predictors and were not included in all four final logistic regression models. No variables met the criteria for significant confounding and none of the interaction terms tested was significant. Results of likelihood-ratio tests were highly significant ($P < 0.001$) for all four final models, suggesting that the variables did contribute significantly to the prediction of shell disease development. Results of the Pearson chi-square and Hosmer-Lemeshow tests for all four models were not statistically significant ($P > 0.05$) indicating that there was no evidence that the data did not fit the models. The Pearson standardized residuals were inspected and showed no obvious outliers and examination of the leverage and delta-betas showed no influential covariate patterns in any of the models.

The final models are shown below (Table 2) as coefficients relative to the log odds of shell disease development. Table 3 shows the logistic regression analysis of shell disease development as odds ratios (ORs) for shell disease development at the four time points.

At all time periods, low [TP] emerged as the strongest predictor of shell disease, with the odds of developing shell disease by 90 days being 26.78 times greater in low [TP] lobsters compared with high [TP] lobsters, the referent category. Despite a moderate correlation with [TP] ($r = 0.61$), intermolt stage remained as a significant predictor at 30 and 90 days, with marginal significance at 60 and 120 days. Sludge removal, in comparison, emerged as a significant predictor in the two final models at 90 and 120 days (and marginal significance at 30 and 60 days), with the odds of developing shell disease being greater in rinsed lobsters compared with the unrinsed lobsters. Having a soft/medium shell, compared with a hard shell, only significantly contributed to the development of shell disease at 30 days, with marginal significance at 90 days as well.

DISCUSSION

Shell disease development in captive populations of lobsters has typically been associated with poor husbandry (Sindermann 1989, Getchell 1991) resulting in exoskeletal damage. In this study, however, we saw that shell disease can occur with high cumulative incidences in the absence of obvious physical damage to the shell. This supports the belief that mechanical trauma is not a necessary prerequisite for lesion development, and other factors, such as poor physiological or nutritional condition (as indicated by low [TP] in hemolymph), increase a lobster's susceptibility to developing shell disease (Bullis 1989).

Our study showed that the physiological differences in groups of lobsters can lead to differences in incidence of shell

TABLE 1.

Averages for total protein concentration ([TP]) (mg mL^{-1}; mean ± SD) and shell hardness (Shell) (durometer units; mean ± SD) in American lobsters ($n = 540$) prestorage, by dealer (midshore, inshore), molt stage (premolt, intermolt), and sex (male, female).

	Intermolt		Premolt		Male		Female		Total	
Midshore:										
[TP]	**38.3**	(**14.6**)[1]	**69.4**	(**17.2**)[1]	44.9	(20.0)[1]	43.9	(19.0)[1]	44.4	(19.6)[1]
Shell	**86**	(**5.8**)[1]	**91**	(**3.2**)	**86**	(**5.9**)[1]	**88**	(**5.3**)	87	(5.7)[1]
Inshore:										
[TP]	**53.8**	(**18.9**)[2]	**78.6**	(**15.6**)[2]	64.4	(20.0)[2]	60.7	(22.8)[2]	62.7	(21.4)[2]
Shell	**88**	(**5.0**)[2]	**91**	(**2.6**)	89	(4.0)[2]	89	(4.9)	89	(4.4)[2]
Total:										
[TP]	**44.7**	(**18.2**)	**75.1**	(**16.8**)	54.0	(22.2)	51.7	(22.5)	53.0	(22.4)
Shell	**87**	(**5.6**)	**91**	(**2.9**)	88	(5.3)	89	(5.2)	88	(5.2)

Significant ($P < 0.05$) differences between molt stages and sexes are indicated in bold. Significant ($P < 0.05$) differences between midshore and inshore lobsters are indicated with different superscripts.

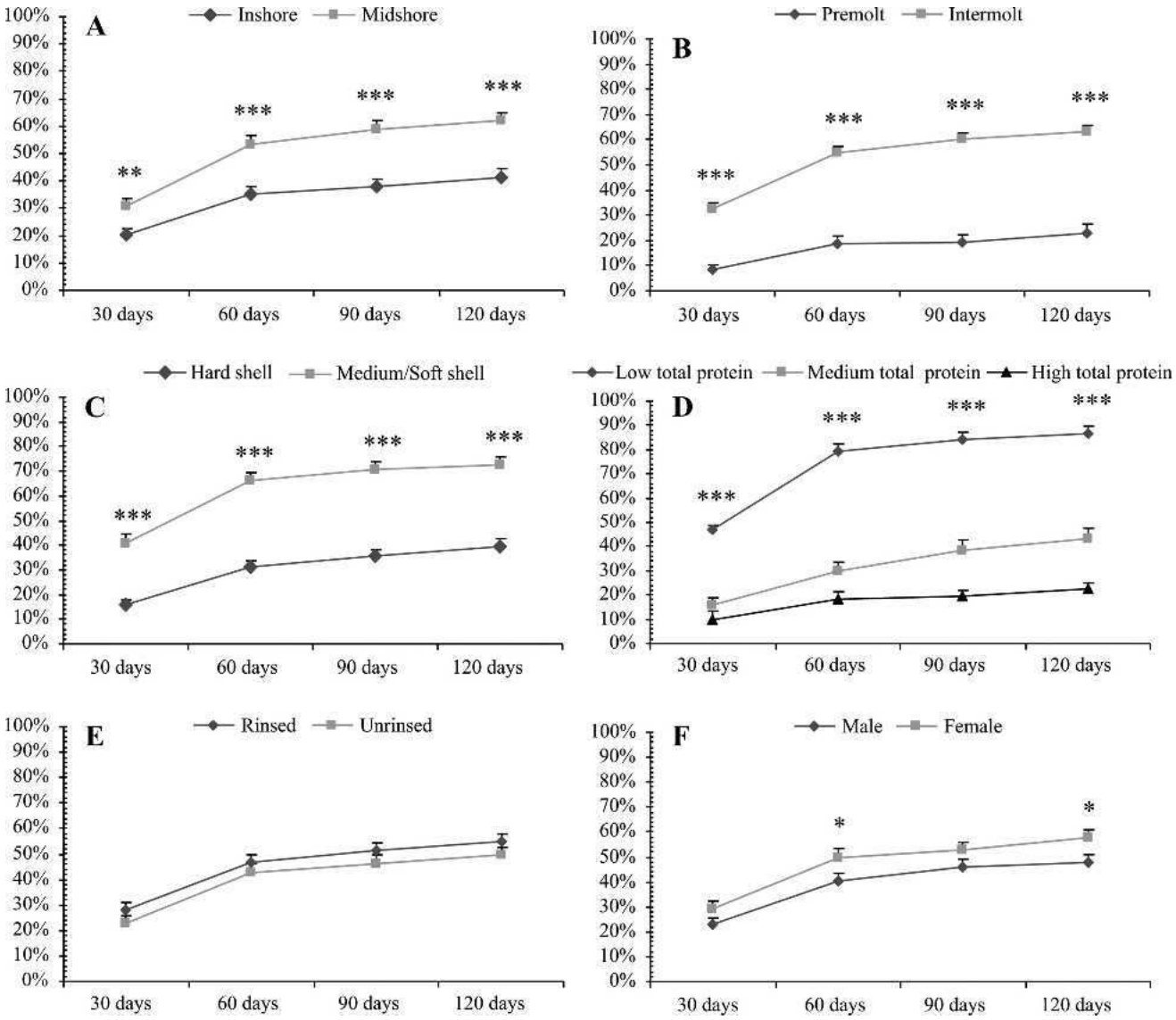

Figure 1. Shell disease % cumulative incidence in impounded American lobsters ($n = 540$) at each sampling period, by dealer (A), molt stage (B), shell hardness (C), total protein concentration (D), wash (E) and sex (F) categories. Error bars show standard error of the mean. Statistical differences are indicated by asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

disease development. [TP] emerged as the main indicator of this difference, with the odds of developing shell disease being as much as 27 times higher for lobsters with low [TP] *versus* lobsters with high [TP]. This study fulfilled several of the necessary criteria for establishing a causal relationship between [TP] and the development of shell disease, including temporality of shell disease measurements (low [TP] was measured prior to the development of shell disease) and a dose:reponse relationship between [TP] and shell disease (as [TP] decreased, the odds of developing shell disease increased). There is also biological plausibility to this discovered association. Low [TP] can 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). Whereas the association between reduced [TP] and poor physiological condition has been established, previous efforts to develop predictive models based on this factor have been unsuccessful (Prince 1997). Our study showed strong evidence that low [TP] is one of the best risk factors in predicting shell disease development in an impoundment facility. The only remaining causality 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 [TP] on entry into the storage facility. Whereas studies have shown that the [TP] of lobster hemolymph varied directly with the lobster's nutritional condition and muscle content (Stewart et al. 1967, Stewart et al. 1972) the molting cycle, and the physiological changes associated with this cycle, assert the largest impact on hemolymph total protein concentrations (Stewart & Li 1969, Barlow & Ridgway 1969). [TP] levels increase during the premolt stage, presumably because of

TABLE 2.

Logistic regression final models at 30, 60, 90, and 120 days.

30 d:	$\ln\{p/(1-p)\}^{a} = -2.62 + 0.17\text{Medium [TP]}^{b} + 1.43\text{Low [TP]}^{b} + 0.73\text{Molt}^{c} + 0.47\text{Shell}^{d} + \in$
60 d:	$\ln\{p/(1-p)\} = -1.81 + 0.83\text{Medium [TP]} + 3.07\text{Low [TP]} + \in$
90 d:	$\ln\{p/(1-p)\} = -2.72 + 1.01\text{Medium [TP]} + 3.48\text{Low [TP]} + 0.86\text{Molt} + 0.51\text{Rinse}^{e} + \in$
120 d:	$\ln\{p/(1-p)\} = -1.63 + 0.80\text{Medium [TP]} + 3.31\text{Low [TP]} + 0.66\text{Rinse} + \in$

[a] p = the cumulative incidence of shell disease.
[b] Coefficients for Medium total protein concentration ([TP]) and Low total protein concentration ([TP]) indicate log odds relative to the High total protein.
[c] Coefficient for Molt stage (Molt) indicates log odds of intermolt lobsters relative to premolt lobsters.
[d] Coefficient for Shell hardness (Shell) indicates log odds of soft/medium shelled lobsters relative to hard shelled lobsters.
[e] Coefficient for Rinse indicates log odds of sludge being rinsed off the lobsters relative to unrinsed lobsters.

a resorption of protein from the chitin-protein complex of the old exoskeleton, and drop dramatically immediately postmolt caused by dilution of the blood by water absorption (Barlow & Ridgway 1969).

In southwest Nova Scotia, the timing of molting (ecdysis) is influenced by variations in environmental conditions (Waddy et al. 1995) but generally occurs during the summer and early fall. Results of our pleopod staging were consistent with this pattern, with 73% of the lobsters in this study in intermolt stage (pleopod stage 0). The remaining 27% of the lobsters were in the premolt stage, but they exhibited only the initial stages of epidermal retraction from the cuticle (pleopod stages 1.0–2.5). These stages likely represent lobsters, which had not yet molted or, alternatively, had molted earlier in the season and were preparing for their next molt. Whereas it is possible for lobsters to continue to develop from pleopod stage 0 up to pleopod stage 2.5 during the winter or at cold water temperatures, they will not proceed beyond these initial stages until the water warms up in the spring (Aiken 1973).

A lobster's molt stage emerged as an important predictor for shell disease in our study, with the odds of developing shell disease being higher in the intermolt lobsters compared with the premolt lobsters in the 30 and 90 day models, and a trend towards a relationship in the 60 and 120 day models as well. Because intermolt lobsters are more susceptible to shell disease, the timing of the fishery in southwest Nova Scotia in relation to the molt cycle might explain the variability in shell disease seen in impoundment facilities year to year. Cooler water temperatures can cause a delayed molt or a delayed recovery from the molt and result in poorer quality lobsters being observed at the time of harvest in late November. Because the southwest Nova Scotia fishery is dominated by fall landings, with an average of 48% of the catch occurring in the first four weeks of the season (DFO 2001), a poorer quality catch during this period can have a significant economic impact on the lobster industry.

Dealer did not emerge as a significant predictor of shell disease in the final models. However, our results did show a significant difference in [TP] between the two dealers. Specifically, lobsters from the dealer who obtained lobster primarily from midshore grounds had lower [TP] and shell hardness levels, particularly in the intermolt lobsters, compared with the primarily inshore lobster from the other dealer (see Table 1). Therefore, molt stage, [TP] and hardness are likely to be intervening variables (Dohoo et al. 2003) for the dealer-shell disease relationship. Therefore, the variation in shell disease related to dealers is likely partially accounted for by these measured variables in the final models, preventing dealer from remaining in the final models.

These results support the industry belief that lobsters from midshore grounds are generally of poorer quality (as indicated in this study by lower [TP] and shell hardness levels) as compared with lobsters from the more inshore grounds. Further examination of this difference is important as fishing effort continues to increase in this midshore area of Nova Scotia, which by the late 1990s accounted for 20% to 30% of the total landings for LFA 34 (DFO 2001). A cooperative study is currently underway, which is examining this difference in lobsters as well as molt timing, lobster quality, and environmental conditions for both inshore and midshore grounds in southwestern Nova Scotia (Retzlaff et al. 2007). To date, this study has shown that the lobsters from the midshore or deeper water sites had lower blood protein levels as well as a later molt. This was attributed to a slower water warming trend observed

TABLE 3.

Odds ratios (±SE) of development of shell disease in impounded American lobsters ($n = 540$) for final logistic regression models at 30, 60, 90, and 120 days.

	30 days	60 days	90 days	120 days
Medium [TP]	1.18 (0.43)	1.86 (0.57)**	2.63 (0.95)**	1.86 (0.59)*
Low [TP]	4.29 (1.48)**	16.08 (5.32)**	26.78 (10.06)**	21.12 (7.36)**
Intermolt	2.07 (0.78)**	1.60 (0.50)*	2.28 (0.87)**	1.54 (0.50)*
Rinse	1.47 (0.32)*	1.35 (0.29)*	1.67 (0.41)**	1.94 (0.45)**
Soft/Medium shell	1.62 (0.39)**	1.14 (0.29)	1.47 (0.38)*	1.05 (0.27)

Significance levels are indicated by asterisks (*$P < 0.20$; ** $P < 0.05$).

in the spring in the midshore locations, as compared with the inshore locations.

Our study suggested that sex was not a significant predictor for shell disease in the final model, which is contrary to previous studies of natural populations where females, especially ovigerous females, have generally demonstrated higher shell disease prevalence (Ziskowski et al. 1996, Young 1991, Baross et al. 1978, Estrella 1991). Female lobsters did have slightly lower [TP], but not significantly different than male lobsters. Perhaps females are more susceptible to the natural manifestation of shell disease (burn spot and epizootic lobster shell disease) but not the form that manifests in impounded lobsters.

Shell hardness was a significant moderate (OR = 1.6) predictor in the 30 day model only, suggesting that this factor might be somewhat important early in the storage period. However, because lobster pound storage times generally exceed 30 days, it seems that shell hardness is not a useful indicator of long-term storage performance compared with [TP], even though it is less invasive than determining [TP] in the hemolymph. Shell hardness and [TP] were moderately correlated ($r = 0.52$), and therefore the variation in shell disease related to shell hardness was likely partially accounted for by [TP] in the final models, perhaps preventing hardness from remaining in the final models for 60 days, 90 days, and 120 days.

The effect of rinsing the lobsters showed unexpected results in the logistic regression models, with the odds of developing shell disease being lower for those lobsters on which the sludge was allowed to accumulate. This protective benefit appeared to be a cumulative effect over time, with the highest odds ratio for rinsing seen in the 120 day model. These results should be interpreted with caution, however, because it is unclear whether the increased susceptibility was caused by the absence of the sludge or simply a side effect of increased stress from the rinsing process. Without specific data on sludge levels, characteristics, and composition, a discussion of criteria for causality would be premature, but sludge represents an interesting area for future research.

Our study reinforces the fact that the quality of the lobsters entering a long term storage facility is critical. Whereas eliminating shell disease altogether is unlikely, our study shows that monitoring the quality of the lobsters and minimizing the length of times in storage for those lobsters, which are more susceptible to developing shell disease, could help to reduce the incidence of shell disease within a long term storage facility. Further efforts to reduce the amount of stress exposure on lobsters post harvest through improvements in the handling or physical treatment of the lobsters, as well as improvements in the environmental conditions experienced during storage, would also likely help to reduce the development of shell disease in long term impoundment facilities.

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LITERATURE CITED

Aiken, D. E. 1980. Molting and growth. In: J. S. Cobb & B. F. Phillips, editors. The biology and management of lobsters. Vol 1. New York: Academic Press. pp. 91–163.

Aiken, D. E. 1973. Proecdysis, setal development, and molt prediction in the American lobster, *Homarus americanus*. *J. Fish. Res. Board Can*. 30:1337–1344.

Barlow, J. & G. Ridgway. 1969. Changes in serum protein during the molt and reproductive cycles of the American lobster, *Homarus americanus*. *J. Fish. Res. Board Can*. 26:2101–2109.

Baross, J. A., P. A. Tester & R. Y. Morita. 1978. Incidence, microscopy, and etiology of exoskeleton lesions in the Tanner crab, *Chionoectes tanneri*. *J. Fish. Res. Board Can*. 35:1141–1149.

Bullis, R. A. 1989. Shell disease in impounded American lobsters, *Homarus americanus*. *Biol. Bull*. 177:327.

Bullis, R., L. Leibovitz, L. Swanson & R. Young. 1988. Bacteriologic investigation of shell disease in deep sea crabs, *Geryon quinquedens*. *Biol. Bull*. 175:304.

DFO. 2001. Southwest Nova Scotia (Lobster Fishing Area 34). DFO Sci. Stock Status Rep. C3-62.

Dohoo, I., W. Martin & H. Stryhn. 2003. Ecologic and Group Level Studies. In: S. McPike, editor. Veterinary epidemiology research. Charlotteteown: AVC Inc. pp. 18–24.

Estrella, B. 1991. Shell disease in the American lobster, *Homarus americanus*, from Massachusetts coastal waters with considerations for standardizing sampling. *J. Shellfish Res*. 10:483–488.

Fisher, W. S., T. Rosemark & E. H. Nilson. 1976. The susceptibility of cultured American lobsters to a chitinolytic bacterium. *Proc. World Maricult. Soc*. 7:511–520.

Foyle, T., G. Hurley & D. Taylor. 1989. Field testing shell hardness gauges for the snow crab fishery. *Can. Ind. Rep. Fish. Aquat. Sci*. No. 193.

Gallager, M., W. Brown, D. Conklin & M. Sifri. 1978. Effects of varying calcium/phosphorous ratios in diets fed to juvenile lobsters, *Homarus americanus*. *Comp. Biochem. Physiol*. 60:467–471.

Getchell, R. G. 1989. Bacterial shell disease in crustaceans: A review. *J. Shellfish Res*. 8:1–6.

Getchell, R. G. 1991. Lobster shell disease survey. *J. Shellfish Res*. 10:489–490.

Goarant, C., R. Brizard & A. L. Marteau. 2000. A white spot disease-like syndrome in the Pacific blue shrimp, *Litopenaeus stylirostris*, as a form of bacterial shell disease. *Aquaculture* 183:25–30.

Hess, E. 1937. Shell disease in lobsters, *Homarus americanus*, caused by chitinivorous bacteria. *J. Biol. Board Can*. 3:358–362.

Hicks, D. & B. Johnson. 1987. Calibration of a meter that measures the shell hardness of Dungeness crab, *Cancer magister*, with an application to commercial landings. Alaska Department of Fish and Game. M88-30-2. Kodiak, Alaska.

Hosmer, D. & S. Lemeshow. 1989. Applied Logistic Regression. New York: Wiley and Sons.

Kapareiko, D., R. Robohm, J. Ziskowski, G. Sennefelder & A. Calabrese. 2001. Shell disease prevalence in offshore American lobster populations. Twenty-sixth Annual Eastern Fish Health Workshop, Kearneysville, WV.

Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. In: S. Colowick & N. Kapalan, editors. Methods in enzymology. Vol 3. New York: Academic Press. 450 pp.

Lightner, D. V., B. Hunter, P. Margarelli & L. Colvin. 1979. Ascorbic acid: nutritional requirement and role in wound repair in penaeid shrimp. *Proc. World Maricult. Soc*. 10:513–528.

Malloy, S. C. 1978. Bacteria induced shell disease of lobster, *Homarus americanus*. *J. Wildl. Dis*. 14:2–10.

Prince, D. L. 1997. Studies on the etiology and pathogenesis of shell disease in the American lobster, *Homarus americanus*. Doctoral dissertation, University of Maine, Orono, ME.

Prince, D. L., R. C. Bayer, M. L. Gallagher & M. Subramanyam. 1995. Reduction of shell disease with an experimental diet in a Nova Scotia lobster pound. *J. Shellfish Res*. 14:205–207.

Retzlaff, A., R. Claytor, B. Petrie, C. Frail, J. Tremblay, D. Pezzack & J. Lavallée. 2007. Variation in molt timing and market quality in the American lobster (*Homarus americanus*). Bedford Institute of Oceanography: 2006 in Review. Cat. No. Fs101-3/2006E, ISBN: 978-0-662-46177-7, ISSN: 1499-9951. pp. 22–26.

Sindermann, C. J. 1991. Shell disease in marine crustaceans: A conceptual approach. *J. Shellfish Res*. 10:491–494.

Sindermann, C. J. 1989. The shell disease syndrome in marine crustaceans. NOAA Technical Memorandum NMFS-F/NEC-64.

Smolowitz, R., A. Hsu, E. Summers & A. Chistoserdov. 2002. Lesions associated with recent epizootic shell disease in *Homarus americanus* on the northeast coast. *J. Shellfish Res*. 21:412.

Smolowitz, R., R. A. Bullis & D. A. Abt. 1992. Pathologic cuticular changes of winter impoundment shell disease preceding and during intermolt in the American lobster, *Homarus americanus*. *Biol. Bull*. 183:99–112.

Statacorp. 1999. Stata Statistical Software: Release 6.0. Station, TX: Stata Corporation.

Stewart, J., G. Horner & B. Arie. 1972. Effects of temperature, food, and starvation on several physiological parameters of the lobster, *Homarus americanus*. *J. Fish. Res. Board Can*. 29:439–442.

Stewart, J. & M. Li. 1969. A study of lobster, *Homarus americanus*, ecology using serum protein concentration as an index. *Can. J. Zool*. 47:21–28.

Stewart, J., J. Cornick, D. Foley, M. Li & C. Bishop. 1967. Muscle weight relationship to serum proteins, hemocytes, and hepatopancreas in the lobster, *Homarus americanus*. *J. Fish. Res. Board Can*. 24: 2339–2354.

Van Olst, J., J. Carlberg & J. Hughes. 1980. Aquaculture. In: J. Cobb & B. Phillips, editors. The biology and management of lobsters. Vol. 1. New York: Academic Press. pp. 333–378.

Vogan, C. L. & A. F. Rowley. 2002. Dynamics of shell disease in the edible crab, *Cancer pagurus*: A comparative study between two sites on the Gower Peninsula, South Wales, UK. *Dis. Aquat. Organ*. 52:151–157.

Waddy, S. L., D. E. Aiken & D. P. V. de Kleijn. 1995. Control of growth and reproduction. In: J. Factor, editor. The biology of the lobster. New York: Academic Press. pp. 217–266.

Young, J. S. & J. B. Pearce. 1975. Shell disease in crabs and lobsters from the New York Bight. *Mar. Pollut. Bull*. 6:101–105.

Young, R. 1991. Prevalence and severity of shell disease among deep sea red crabs (*Chaceon quinquedens*, Smith, 1879) in relation to ocean dumping of sewage sludge. *J. Shellfish Res*. 10:499–503.

Ziskowski, J., R. Spallone, D. Kapareiko, R. Robohm, A. Calasbrese & J. Pereira. 1996. Shell disease in American lobster in the offshore Northwest-Atlantic region around the 106-mile sewage sludge disposal site. *J. Mar. Environ. Eng*. 3:247–271.