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**RISK FACTORS AFFECTING POST-CAPTURE HEALTH
AND PRODUCTIVITY OF IMPOUNDED AMERICAN LOBSTERS
(*HOMARUS AMERICANUS*) IN ATLANTIC CANADA**

A Thesis
Submitted to the Graduate Faculty
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
in the Department of Health Management
Faculty of Veterinary Medicine
University of Prince Edward Island

Jean Lavallée
Charlottetown, P.E.I.
April, 1999

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ABSTRACT

The Canadian lobster industry holds lobsters (*Homarus americanus*) in captivity for various periods to supply the market with live product year-round. Mortalities during holding result in considerable losses, estimated at 10 to 15% per year by the industry. The first part of this study evaluated lobster health variation among fishing boats from different fishing ports located in New Brunswick, Nova Scotia and Prince Edward Island from May 1996 to February 1997. A total of 3,525 lobsters from 99 different boats were tagged and monitored. Significant differences ($P \leq 0.05$) were observed in lobster weight, carapace length, sex, overall physical condition and total haemolymph protein (THP) among the fishing ports, and among fishing boats from common ports. The overall proportion of lobsters with normal physical condition significantly decreased from 76.1% before holding to 43.6% after holding ($P < 0.001$). Significant increases in THP from before to after impoundment were observed with averages of 11.7 g/L and 18.8 g/L after holding ($P < 0.001$ and $P = 0.007$ respectively). Biomass variations during impoundment ranged from a decrease of 0.06% per week to an increase of 0.05% per week, while the observed mortality rates varied between 0% per week to 1.09% per week. The second part of the study assessed lobster fishing practices on various boats, and related these practices to lobster vigour measured at the processing plants. A total of 2,191 lobsters landed from 64 boats in 1997 were included in the study. Lobsters were tagged and their vigour assessed on the boats, at the wharves, and at the time of entry into the processing plants, while fishing and transportation practices were monitored. Significant increases ($P \leq 0.05$) ranging between 3.1% to 10.2% in the proportion of lobsters with open wounds from the time of capture to the time of entry in the processing plant were found. THP levels ($P < 0.001$) and total haemocyte counts ($P < 0.001$) were linearly associated with the time of the year. A generalized estimating equation was used to assess the impact of handling, fishing and transportation practices on lobster vigour measured upon arrival at processing plants. Significant risk factors for lobster loss of vigour included the following boat-level factors: the use of mackerel for bait with an odds ratio (OR) of 7.1 ($P = 0.003$), tossing lobsters from traps to temporary holding units on the fishing boats (OR=3.6, $P = 0.048$), exposure to rain on the fishing boats (OR=3.6, $P = 0.011$), while the maximal depth at which the traps were set had a protective effect on lobster vigour (OR=0.85/m, $P = 0.010$). The third part of this study examined the prevalence of *Anophryoides haemophila* and *Aerococcus viridans*, causative agents of bumper car disease and gaffkemia respectively, in lobsters caught in the waters of Prince Edward Island during the spring and fall fishing seasons of 1997. A total of 116 lobsters were sampled in the spring, and 138 in the fall. *A. haemophila* was not detected in the spring, while the prevalence was 0.72% in the fall with a 95% confidence interval (CI) of 0.02%-3.97%, and an overall prevalence of 0.39% (95% CI: 0.01%-2.17%). The prevalence of *A. viridans* was estimated at 6.9% (95% CI: 3.02%-13.14%) in the spring, 5.8% in the fall (95% CI: 2.54%-11.10%), and 6.30% overall (95% CI: 3.64%-10.03%).

DEDICATION

“À mes parents Claudette et Gilles, à ma soeur Josée, son mari Serge et leur merveilleuse petite fille Jade. Merci pour votre amour, votre soutien et surtout pour avoir cru en moi. Merci pour tout . . .”

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TABLE OF ABBREVIATIONS

ANOVA	: Analysis of variance
ASW	: Artificial seawater
95% CI	: 95 percent confidence interval
CL	: Carapace length (in mm)
cm	: Centimetre
°C	: Degree Celsius
df	: Degrees of freedom
DFO	: Department of Fisheries and Oceans, Canada
g	: Gram
GEE	: Generalized estimating equation
h	: Hour
kg	: Kilogram
L	: Litre
lbs	: Pound
mm	: Millimetre
mt	: Metric ton
n	: Number of observations
na	: Not available
nd	: Not done
OR	: Odds ratio
P	: Probability of type I error
PEA	: Phenylethylalcohol
PEI	: Prince Edward Island
PO	: Phenoloxidase
ProPO	: Prophenoloxidase
%	: Percent
r	: correlation coefficient
RR	: Relative risk
SD	: Standard deviation
SE	: Standard error
SEM	: Standard error of the mean
THP	: Total haemolymph protein
THC	: Total haemocyte counts
wk	: Week
wt	: Weight

1. INTRODUCTION

The studies reported in this thesis attempt to quantify lobster (*Homarus americanus*) industry losses during periods of live-storage in all three Maritime provinces and assess different factors which may have contributed to these losses by examining associations with wharf and fishing boat factors. By assessing lobster health and by following individual lobsters through the different handling points of the Atlantic Canadian industry, the points where post-harvest losses occurred were identified. These points included fishing boats, wharves, transport, and holding facilities or processing plants. The effect of different fishing and handling practices on the selected indices of lobster health and quality was also investigated, and discussed with relationship to lobster biology.

Additionally, the prevalence of the infectious agents that cause the major diseases of concern to the post-harvest industry was estimated in lobsters freshly-caught from the fishing areas of coastal Prince Edward Island, Canada.

1.1 Canadian lobster industry

The Canadian lobster fishery represents one of the last sustainable fisheries in the Maritime provinces (TAVEL Report, 1995). It is an important component of the inshore fishery, directly employing over 25,000 people. This includes 16,000 licensed fishers and more than 7,000 others working in approximately 250 processing plants and exporting facilities (TAVEL Report, 1995). After reaching record landings in 1991 with more than 48,000 metric tons

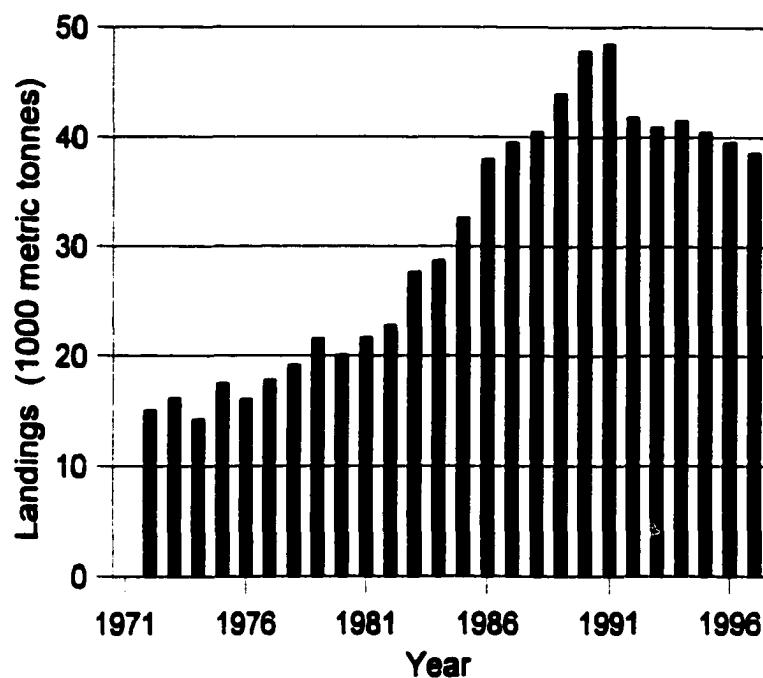


Figure 1. Commercial landings of American lobsters (*Homarus americanus*), live weight, in Canada from 1972 to 1996 (Source: DFO, 1999).

(mt), the weight of lobsters landed in Atlantic Canada appears to have stabilized at approximately 40,000 mt each year since 1992 (Fisheries and Oceans Canada, 1999) (Figure 1). The east coast of Canada is divided into almost 40 different lobster fishing areas, or LFAs (Miller, 1995). Each of these LFAs is managed according to its own characteristics (local environmental conditions and lobster life history) and they differ from each other by having different sized areas, fishing seasons, minimum legal carapace sizes, maximum trap numbers, numbers of licensed fishers per LFA, and numbers of open fishing days (Pringle and Burke, 1993; Miller, 1995).

On average, nearly half of the Canadian landings are transformed into processed products, while the rest are destined to be sold alive, (TAVEL Report, 1995). Most often, fishers will sell their daily catch to buyers situated at the wharves. These buyers can be independent dealers or representatives of other seafood companies. The buyers will either transport the live lobsters directly to the processing plant or to a holding facility; this transfer usually occurs within one to five days post-capture. If it is decided to keep the lobsters for a few days, they will either be kept in wooden crates and floated directly at the wharf, or stored in 'lobster cars', which are large floating boxes (approximately 10 m x 10 m x 2 m deep) also located at the wharf. Lobsters can either be loose in the cars, or stored in crates within the lobster car. To supply the market with a live product year-round, some of the processing plants and holding facilities will keep a proportion of live lobsters in captivity for various periods, ranging from a few days to twelve months.

There are five different types of holding facilities used in Atlantic Canada for short- to long-term impoundment of live lobsters (Pringle and Burke, 1993). These include tidal pounds, dryland pounds, tank houses, inside pounds or reservoir holdings, and lobster cars. A tidal pound is an open coastal system where lobsters are housed in fenced-in areas, or sometimes part of a bay, where the flushing action of the tides supplies the pound with 'new' water twice daily. Tidal pounds typically have the capacity to hold from 40,000 to more than 100,000 kg of live lobster at a time. Dryland pounds are recirculating systems with filtered and chilled (<3 °C) water, where lobsters are held individually in compartmentalized trays. A typical dryland pound can have a capacity of more than 900,000 kg. Both dryland and tidal pounds are mostly used for long term holding, i.e., from a few weeks to several months. Tank houses consist of a series of fibreglass, wooden, or plastic tanks with either a flow-through supply of water or recirculated water. The water can be chilled, and capacities vary from a few thousand to up to 45,000 kg. An inside pound or reservoir holding refers to an open system with large concrete tanks where lobster crates are stored from a few days to a few weeks, with capacities sometimes reaching 12,000 kg of live lobster. The fifth type of holding is the lobster car, usually located near a wharf, or anchored at a short distance from the shore, and consisting of a large box that can hold 400 to 2,000 kg of live lobsters. Lobster cars are designed for short-term holding only (usually less than 7 days); they lack any movement of water other than the natural currents where they are located, unless a mechanical aerator is placed underneath the holding system.

1.2 Lobster life cycle

Mating usually occurs shortly after the female lobster molts at which time she is in a soft-shelled condition (Phillips *et al.*, 1980; Talbot and Helluy, 1995). The eggs are expelled from the oviducts through a gonopore located at the base of the third pair of walking legs, and remain attached for the next 9 to 12 months (Talbot and Helluy, 1995). The embryos will molt several times within the eggs, will then hatch and the prelarvae will eventually be released by rhythmic fanning of the female's pleopods (Talbot and Helluy, 1995). The prelarvae will molt into the first larval stage, at which time they are found near the water surface during daylight, followed by metamorphosis into postlarvae at the fourth molt (Talbot and Helluy, 1995; Lavalli and Lawton, 1996). As they grow larger (average carapace length of 25 to 40 mm), the juveniles explore and forage further away from their burrows (Lavalli and Lawton, 1996). Adolescent lobsters are of considerable size (carapace length of about 50 mm), but are not yet sexually mature (Lavalli and Lawton, 1996). Sexual maturity is reached after five to eight years, depending mainly on the water temperatures and gender; males will usually mature at a smaller size than females (Talbot and Helluy, 1995; Lawton and Lavalli, 1995). For a schematic representation of the external anatomy of an adult lobster, see Appendix A.

1.2.1 Molting and growth

The shell of the lobster is hard and inflexible and must be shed periodically through the process of ecdysis, to allow for growth. Premolt is

induced endocrinologically, and triggered by internal and environmental signals such as water temperature (Aiken and Waddy, 1992). Mineral components of the old exoskeleton are redistributed into large gastroliths (discoid calcareous nodules) on either side of the cardiac stomach wall (Waddy *et al.*, 1995). Additionally, a proportion of haemolymph is withdrawn from the appendages to make them smaller; Mykles (1992) reported 30 to 60% loss of tissue mass in the claws, facilitating the withdrawal of the large portion of the claw through the smaller upper leg portion through which it must pass. Prior to ecdysis, lobsters ingest substantial volumes of seawater. This forces the new exoskeleton to expand and pushes apart the old shell (Waddy *et al.*, 1995). Even the gills, mouthparts, antennae, antennules, eyestalks, and pleopods are withdrawn, and the cuticular lining of the digestive system is shed resulting in the gastroliths falling into the stomach and being dissolved by the digestive fluids (Waddy *et al.*, 1995). The new-shelled lobster immediately resumes water uptake and also pumps water through its branchial chambers at a maximal rate; the body fluid volume can be approximately 50% greater than before ecdysis (Waddy *et al.*, 1995).

1.2.2 Molt stages

The molt cycle can be differentiated into a series of five stages from A to E, based on physiological and integumentary changes. Stage A (post-molt) starts immediately after the completion of ecdysis, and accounts for approximately 2% of the entire molt cycle, while stage B (also considered post-

molt) starts with the deposition of the endocuticle and accounts for 8% of the cycle (Aiken and Waddy, 1992). Stage C lasts up to 65% of the molt cycle and begins when the changes in the epicuticle and exocuticle are completed (Aiken and Waddy, 1992). Stage C is divided into four substages, with C_3 being the last phase of the 'post-molt,' while C_4 represents the 'intermolt' phase and it is associated with the completion of the cuticle and final ovary maturation and spawning (Waddy *et al.*, 1995). Stage D is divided into five sub-stages, and is referred to as the premolt or proecdysis stage and accounts for at least 24 to 75% of the molt cycle, depending on the species (Aiken and Waddy, 1992). Stage D starts with the retraction of the epidermis from the old cuticle (apolysis) and ends with the opening of the cephalo-thoraco-abdominal membrane. Finally, stage E occurs and it consists of the ecdysis itself, which rarely lasts more than 30 minutes (Waddy *et al.*, 1995).

1.2.3 Components of the immune system

The components of the crustacean immune system are divided into three major categories; *physical barriers*, *cellular response* and *humoral response* (Millar and Ratcliffe, 1994) (see Table I). The immune system is dynamic; although these three categories are distinct, components of one category often interact with component(s) from another category. Major differences between the crustacean immune system and the mammalian immune system are the absence of immunoglobulins (or true antibodies) and the absence of true leukocytes (Hall *et al.*, 1972; Söderhäll and Cerenius, 1992). There are three

Table I. Components of the lobster immune system.

DEFENCE REACTION	ROLE IN IMMUNITY
PHYSICAL BARRIER	
exoskeleton	Impenetrable barrier when intact, it may also contain ProPO and a protease inhibitor
CELLULAR RESPONSE	
clotting/coagulation	Prevents loss of haemolymph at the site of injury, seals the open wound and could also immobilize pathogens entering through the site of injury
haemocyte responses	Ingestion of small numbers of microorganisms (phagocytosis), formation of clumps consisting of large number of microorganisms entrapped in several layers of haemocytes (nodule formation), and sequestration of large particles by several haemocytes to seal off the foreign particle from the circulation (encapsulation response)
pinocytosis	Clearance of microorganisms by podocytes in gills and in antennal glands
phagocyte response	Sequestration and phagocytosis of many pathogens by the fixed phagocytes lining the vessels of the hepatopancreas and the gills
HUMORAL RESPONSE	
agglutinins	Recognition of non-self particles and therefore agglutination of these foreign particles and possibly enhancement of their elimination
lysins	Natural protein capable of cell lysis
opsonins	Render bacteria and/or other particles susceptible to phagocytosis
bacteriocidins	Require the release of some material from haemocytes to be activated and have a bactericidal activity
prophenoloxidase (ProPO)	Major role in recognition and elimination of foreign particles from the haemolymph

Adapted from Millar and Ratcliffe, 1994

types of haemocytes for *Homarus americanus*: hyaline haemocyte, semigranular and granular haemocyte (Battistella *et al.*, 1996; Martin and Hose, 1995; Söderhäll and Cerenius, 1992). The haematopoietic tissue of the American lobster consists of a thin layer of tissue that is loosely bound to the dorsal surface of the foregut, and composed of numerous ovoid lobules (Martin and Hose, 1995). Other haematopoietic sites, situated at the base of the rostrum and near the ophthalmic artery, are also present (Millar and Ratcliffe, 1994).

The plasma clotting protein of crustaceans, coagulogen, is analogous to fibrinogen found in vertebrates. Clot formation is initiated via the enzyme transglutaminase released by the lysis of hyaline haemocytes (Martin and Hose, 1995). A very-high-density lipoprotein in the haemolymph of the sand crayfish (*Ibacus ciliatus*) also has some clotting ability (Komatsu and Ando, 1998). If an injury occurs and an opening is created, a clot is rapidly formed in an attempt to seal this wound, preventing further loss of haemolymph.

In all decapods, the exoskeleton contains an inhibitor against proteolytic enzymes and some prophenoloxidase (ProPO), the inactive form of a protein which plays an important role in the elimination of foreign materials (Söderhäll *et al.*, 1996). Phenoloxidase (PO) found in the haemolymph (Söderhäll and Cerenius, 1992; Millar and Ratcliffe, 1994; Söderhäll *et al.*, 1996) is responsible for the deposition of the pigment melanin (Hose *et al.*, 1990; Söderhäll and Cerenius, 1992; Millar and Ratcliffe, 1994; Söderhäll and Thörnqvist, 1997), is involved in the recognition of foreign particles and therefore, is an important component of the response to disease in crustaceans (Millar and Ratcliffe, 1994;

Söderhäll *et al.*, 1996; Söderhäll and Thörnqvist, 1997). The inactivated form of this protein, ProPO, is contained in granulocytes (Hose *et al.*, 1990; Söderhäll *et al.*, 1996). Martin and Hose (1995) reported that some intermediates of melanin production are cytotoxic and may cause the death of invading microorganisms.

1.3 Health management

Health management is a concept used to describe management practices which are designed to prevent disease or stress. This is a general concept geared toward 'productivity improvement' that sometimes can lead to stress and disease due to the frequent manipulation of the animals necessary in the implementation of the health management program. However, the overall trade-off value of health management programs makes it beneficial to implement, and they can be applied to any livestock, including lobsters. Health management programs are well established in other livestock industries, with proven social and economic benefits to producers (Radostits *et al.*, 1994). Successful health management should involve prevention of disease or elimination of risk factors contributing to disease, rather than treatment. Ideally, prevention of lobster disease would be accomplished through selection of healthy lobsters, good water quality management, meticulous grading for optimal health, nutrition, and sanitation. An interaction between the pathogen(s) and environmental influences on the host is needed to cause disease (Martin *et al.*, 1987). In this model, a pathogen can either be considered a separate causal factor of disease, a component of the environment, or even a component of the host.

1.4 Lobster diseases of economic importance

1.4.1 Bacterial infections

Gaffkemia caused by the Gram-positive tetrad-forming coccus, *Aerococcus viridans*, is an important disease in adult lobsters with potential to cause high mortality rates (Stewart and Zwicker, 1974; Stewart, 1975; Johnson *et al.*, 1981; Marks *et al.*, 1992). Another bacterial disease with major economic impact in impounded lobsters is shell disease or chitinolytic disease. Shell disease is usually present during or after winter impoundment and is primarily limited to lobsters caught in the south-western part of Nova Scotia, Canada (Getchell, 1989).

1.4.1.1 Gaffkemia

Gaffkemia is probably the most important disease of impounded American lobsters (*H. americanus*) and European lobsters (*H. gammarus*) (Stewart, 1975). All ages are susceptible. The bacterium, *A. viridans*, which causes gaffkemia, lacks invasive capability and requires breaks in the exoskeleton to infect lobsters, and extremely low numbers of pathogens are sufficient to cause the death of lobsters (Stewart and Zwicker, 1974; Stewart, 1975; Stewart, 1980). The aggressive behaviour of *H. americanus* (Stewart, 1975; Bayer *et al.*, 1993) resulting in damaged appendages, the over-crowding of crates or pounds, and the rough handling by fishers, buyers and employees of holding facilities or processing plants, are all probable risk factors for outbreaks of gaffkemia (Stewart, 1975). The mean time to death for lobsters injected with as few as 10

bacterial cells per kg of body weight is approximately the same as for lobsters injected with a dose 60 million times as great; 16 to 19 days (Stewart, 1975), demonstrating the importance of only selecting lobsters with intact shell for impoundment.

Lobsters cannot control or efficiently respond to infection with *A. viridans* (Stewart and Zwicker, 1974; Marks et al., 1992). Phagocytosis of the bacteria occurs following the introduction of the pathogen into the animal, but it is ineffective and eventually the phagocytes are destroyed (Johnson et al., 1981). The total number of circulating haemocytes decreases dramatically during the course of infection (Stewart, 1975; Johnson et al., 1981). There is no agglutination and the bactericidal ability of the haemolymph deteriorates in the presence of virulent strains of *A. viridans* (Stewart, 1975; Johnson et al., 1981). As haemocyte numbers decline, clotting ability is lost, although coagulogen and other protein levels do not seem to be significantly affected (Stewart, 1975). Additionally, the bacteria compete for glycogen, thus draining the lobster's carbohydrate reserves, while massive impairment of the vital functions of the lobster's hepatopancreas also occurs (Stewart, 1975 & 1980). Stewart (1980) hypothesized that the dysfunction of the hepatopancreas, actively involved in biosynthesis, detoxification, and primary absorption of food, is apparently the key factor in the pathogenesis of gaffkemia. There are no clinical signs for this disease, other than progressive weakness and lethargy (Stewart, 1975). Death can arise from fatal leakage of haemolymph from wounds.

1.4.1.2 **Shell disease**

Cuticular abrasions or mechanical disruptions are required for the establishment of chitinolytic bacteria (Prince *et al.*, 1993b). The species of chitinolytic bacteria most commonly isolated from typical lesions are Gram-negative species from the genera *Vibrio*, *Pseudomonas*, and *Aeromonas* (Prince *et al.*, 1993a). Subsequently, the bacteria will proliferate and, in advanced cases, there will be pitting and erosion of the cuticle with perforation of the shell (Prince *et al.*, 1993a & 1993b; Getchell, 1989.). This can lead to secondary infections of the underlying tissues that cause most mortalities in lobsters with shell disease, although large lesions can also cause mortality during molting from adhesions between the exoskeleton and underlying tissues (Martin and Hose, 1995).

The first line of defence against chitinolytic pathogens consists of the exoskeleton and the tegumental secretions (Martin and Hose, 1995). The proteolipoidal layer, or epicuticle, contains anti-fouling agents and other antimicrobial compounds such as ProPO (Stewart, 1980; Martin and Hose, 1995). If microorganisms start invading this outer layer of the epicuticle, the next line of defence of the lobster is the mineralization of the exocuticle and endocuticle (Stewart, 1980; Martin and Hose, 1995). Mineralization becomes a particularly strong response when microorganisms reach the more permeable area of the un-calcified endocuticle (Getchell, 1989). Haemocytes also migrate to the site of attack to provide numerous defensive functions such as

phagocytosis, formation of pseudomembranes, and activation of the ProPO system to initiate the melanistic response (Martin and Hose, 1995).

Prince *et al.* (1993a) proposed that many conditions, including poor water quality and the presence of bacterial endotoxins found during winter impoundment in tidal pounds, could exacerbate shell disease. During winter months, the water temperature in the pounds is usually below the optimum temperature for lobsters and Prince *et al.* (1993a) also suggested that haemocyte migration to wound sites is retarded at these low water temperatures. Starvation results in gradual decrease of circulating haemocytes and total haemolymph proteins (Prince *et al.*, 1993a). These events surely render lobsters more vulnerable to shell disease, although they are probably not causing the primary foci of shell deterioration.

1.4.1.3 Other bacterial diseases

Injecting lobsters with Gram-negative bacteria such as *Pseudomonas perolens* increases the levels of bactericidin in the haemolymph and inoculation with their endotoxin elevates the phagocytic index of haemocytes (Paterson *et al.*, 1976). An increase in the total number of circulating haemocytes will occur after inoculation with many species of bacteria (Stewart and Zwicker, 1972), with the exception of *A. viridans* (Stewart, 1975; Johnson *et al.*, 1981). At 12 days post-injection with *P. perolens*, a shift toward larger nuclei cells found in the haemopoietic tissue indicates that differentiating cells are released faster than in haemopoietic tissue of normal lobsters (Johnson *et al.*, 1981).

Vibrio spp. have been reported to affect nutritionally stressed, weak or sick lobsters, and should probably be considered opportunistic pathogens (Bower *et al.*, 1994). Presently, no economically important losses have been attributed to an outbreak of vibriosis in Atlantic Canada. However, *Vibrio* spp. are significant pathogens of cultured shrimp, such as the blue shrimp (*Penaeus stylostris*), and the Tiger shrimp (*P. monodon*) and, causing major economic losses every year (Sung *et al.*, 1994; Chanratchakool *et al.*, 1995; Mohney *et al.*, 1997). Consequently, *Vibrio* spp. might induce important losses in impounded lobsters, but no direct link has been established.

1.4.2 Parasitic infections

The protease inhibitor found in the exoskeleton may have an anti-parasitic effect (Häll and Söderhäll, 1983). One disease of impounded lobsters which was only recently recognized as potentially having significant economic impact on the lobster post-harvest industry, is ciliate disease or bumper car disease (Morado and Small, 1995; Speare *et al.*, 1996). This disease is caused by *Anophryoides haemophila* n. sp., an endoparasite of lobsters (Cawthorn *et al.*, 1996).

1.4.2.1 Bumper car disease

The number of circulating haemocytes is decreased during the course of infection with the ciliate *A. haemophila* (Sherburne and Bean, 1991; Bower *et al.*, 1994; Cawthorn *et al.*, 1996). Although some lectins are present and there is a strong agglutination response to the presence of the ciliate, the lobster's

response to this infection appears to be ineffective (Cawthorn, 1997). Similar to gaffkemia, after the level of infection is high enough to cause significant depletion of haemocytes, lobsters infected with *A. haemophila* may 'bleed' to death if wounded (Cawthorn, 1997). Open wounds or breaks in the carapace are likely routes of entry for the ciliates into the lobster, although there is also indication that the ciliates could pass through the thin cuticle of the gills (Aiken *et al.*, 1973; Cawthorn, 1997). All cases of parasitism reported by Aiken *et al.*, (1973) in the winter 1971-1972 occurred at water temperatures below 10 °C. Cases were also observed at water temperatures less than 5 °C (Aiken and Waddy, 1986), leading one to speculate that bumper car disease is mostly exacerbated during winter impoundment periods.

1.4.3 Viral infections

Presently, there are no viral pathogens known to have significant disease impact in impounded lobsters. However, over seven different viruses affecting shrimps and prawns have been isolated and identified (Overstreet, 1986; Boonyaratpalin *et al.*, 1993; Bruce *et al.*, 1993; Lightner *et al.*, 1995). Conceivably, more specific diagnostic work could result in lobster virus isolation and culture, and pathogenic viruses affecting lobsters could be identified. In the shrimp industry, a disease called 'yellow head disease' has inflicted massive losses, especially in Asia. This disease is characterized by necrosis of circulating haemocytes with absence of significant inflammation (Boonyaratpalin *et al.*, 1993). Supamattaya *et al.* (1998) were able to experimentally transmit

white spot syndrome virus (WSSV) from the tiger shrimp to the mud crab (*Scylla serrata*). Based on this, they suggested that the crab could play a role as a viral reservoir. Although viruses have not yet been implicated in mortalities of impounded lobsters, infections may be present but remain undetected. Perhaps the absence of specific lobster cell lines limits abilities to identify viral infections, and the relatively high cost associated with current diagnostic procedures limits the likelihood of detection.

1.4.4 Fungal infections

There are some fungi associated with infection of eggs and or larval stages, but adult lobsters are usually resistant to fungi (Bower *et al.*, 1994; Martin and Hose, 1995). A few deaths from fungal infection have been reported in cultured male lobsters, and the deaths were most likely due to adhesions restricting ecdysis (Stewart, 1980). After contact with β -1,3-glucans, the ProPO system is activated resulting in melanization of the infection site and encapsulation of the foreign agent (Söderhäll and Cerenius, 1992).

1.5 Conclusion

The volume of lobsters landed annually in Atlantic Canada and kept for the live market is approximately 20,000 mt for a corresponding landed value of \$200 million (Can.). However, significant losses occur during the holding period. An important fraction of these losses are directly related to diseases, and some fishing and handling practices may also predispose lobsters to higher risk of

being downgraded or death. Gaffkemia, bumper car disease and shell disease are presently recognized as the three major diseases with economic impact.

2. HEALTH AND SHRINKAGE ASSESSMENT OF IMPOUNDED LOBSTERS IN ATLANTIC CANADA

2.1 Introduction

The lobster (*Homarus americanus*) fishery represents one of the largest contributors to the total volume landed among the Atlantic Canadian fisheries. For the Atlantic Canadian coast in 1996, the volume of lobsters landed was estimated at 39,542 metric tons, fourth behind shrimp, scallop and the Queen crab which had estimated landed volumes of 56,000 mt, 59,500 mt and 65,700 mt, respectively (Fisheries and Oceans Canada, 1999). The corresponding estimated landed values were \$378.5 million for lobsters, followed by \$218.7 million, \$145.4 million and \$89.7 million, for the Queen crab, shrimp and scallop fisheries, respectively (Fisheries and Oceans Canada, 1999). After reaching record landings in 1991 with more than 48,000 mt, the volume of lobsters landed appears to have stabilized at approximately 40,000 mt each year since 1992. On average, about half of the Canadian landings is transformed into processed products, while the other half is destined for live market, and over 80% of the overall production is exported (TAVEL Report, 1995). Typically, lobsters are held either in tidal pounds which consist of dam-enclosed portions of the shoreline, in tanks, or in dryland pounds where lobsters are placed in compartmented trays supplied with cold water (<3 °C). Both tidal pounds and dryland pounds are used for medium to long term holding, while lobsters stored in tanks are usually held for a maximum of three weeks. Several million

kilograms of lobsters are impounded in Atlantic Canada and the state of Maine, USA, every year, and sometimes for more than 6 months (Prince *et al.*, 1993b).

Typically, from the end of August to the end of October, the Canadian industry holds in tidal pounds newly molted, soft-shelled lobsters caught in Maine (referred to as 'Maine shedders') until the Christmas period. This holding can continue until the end of January or the early part of March of the following year. The purpose of this particular impoundment is to harden the carapace and to allow for an increase in the meat quantity and consequently an increase in their economic value.

Lobsters kept in confined areas are most likely more susceptible to disease epidemics, and therefore, pound operators always expect some loss, referred to as shrinkage. Shrinkage can also be defined as the weight of live lobsters going into holding minus the weight of live lobsters coming out. Mortalities usually account for the major part of the shrinkage. However, the slightest variation in weight, either gain or loss, can have major economic impact as more than 100,000 lobsters are typically held in each of these confinement situations. If there are few mortalities during holding combined with an increase in the average lobster body weight after holding, there is no real shrinkage, but rather a positive weight difference. During lobster holding, mortalities due to disease outbreaks may occur, and the bacterial disease gaffkemia (bacterial disease caused by *Aerococcus viridans*) can inflict significant losses, especially during the summer and fall months (Keith *et al.*, 1992, Stewart, 1975).

Other than mortalities from disease, the origin of the lobsters could also have a potential impact on lobster health; the lobster industry strongly believes in the presence of significant variations in the quality of lobsters derived from different boats and landing areas. Different geographic regions, times of the year, water temperatures, and most likely different sources of food and prey present, variations in lobster quality should be observed among fishing ports. These factors do affect plasma protein, molting cycle, and possibly overall health status (Ennis, 1973; Paterson and Spanoghe, 1997). Unless certain lobster boats from the same port are fishing in different fishing grounds, the physiological parameters of the lobsters entering the traps should be similar.

2.2 Objectives

The primary objective of this study was to assess the variation in lobster health and productivity among different fishing ports located in New Brunswick, Nova Scotia and Prince Edward Island (Canada), and among the different fishing boats from these ports. Other objectives were to quantify lobster productivity losses during holding, and to assess some of the factors correlated to these losses during holding (total haemolymph protein, gender, lobster size, infection with *A. viridans*).

2.3 Materials and methods

Physical assessments (see section 2.3.3) were conducted on groups of lobsters coming from a total of 11 different fishing wharves en route to the 8

different holding facilities. Lobsters were reassessed when they exited the holding facilities in three circumstances, and also during holding in one instance. The holding facilities were selected first. Then the investigators went to the wharves indicated by the holding facilities, according to their commitment to buy lobsters on the proposed sampling days. Finally, the fishing boats were selected among those selling to the holding facilities' representative buyers.

2.3.1 Holding facilities

An attempt was made to get different types of medium to long term holding facilities involved in the study. The research was conducted in two tank houses, two dryland pounds, and four tidal pounds located in the Maritime provinces. The selection of the holding facilities was done via a list of volunteer CALPA (Canadian Atlantic Lobster Promotion Association) members.

2.3.2 Sampling groups

Eleven groups of lobsters numbered from 1 to 11 were sampled at eight different holding facilities during this study. Industry willingness to cooperate and severe weather conditions during some sampling days resulted in a variety of monitoring intensities among the sampling groups.

Lobsters were tagged and assessed directly at the wharves for five sampling groups, and at the holding facilities for the other six. After the seventh group, attempts were made to sex, weigh and physically examine every tagged lobster, while haemolymph was obtained from a random subsample (systematic

sampling). However, body weights were not recorded in one sampling group, due to extreme weather conditions which prevented accurate weighing. For details of numbers of lobsters sampled in each group, see Table I.

One group of lobsters was sampled during the holding period, when the pound employees were removing some lobsters for an unanticipated order, before the expected date of the total emptying. Assessments on lobsters leaving the pound were normally done after the anticipated holding period, when the pounds were emptied. Lobster mortalities in tidal pounds were monitored by scuba divers on a weekly basis for the mortality rate estimation after holding. Adverse weather conditions at some sampling sites prevented complete assessments, and resulted in incomplete data. Furthermore, some data were lost during processing, because of circumstances beyond the control of the investigators; tagged lobsters in some holding facilities were processed and marketed without notice being given to the investigators. Data after the impoundment period were obtained in five lobster pounds.

2.3.3 Fishing boats and lobster selection

An average of ten fishing boats were sampled at each wharf, with one or two crates of lobsters selected per boat (typical wooden crates have holding capacities of 40-50 kg of live lobsters). The fishing boats were selected on a convenience basis, eg., the first ten boats to dock while the investigators were at the wharf. Lobster crates from the selected boats were randomly selected.

Table I. Summary of the sampling protocol for the number of tagged and sampled lobsters for each sampling group, with corresponding holding time.

SAMPLING GROUP	DATE OF INITIAL SAMPLING	HOLDING TIME	# OF BOATS	# TAGGED LOBSTERS	# LOBSTERS SAMPLED						
					SEX	WEIGHT	CL ^e	PHYSICAL CONDITION	THP ^f	AEROCOCCUS VIRIDANS ^g	
24	1 ^a	09/05/96	na	9	92	92	0	92	92	92	92
	2 ^a	16/05/96	na	9	82	82	55	82	82	82	82
	3 ^a	30/05/96	na	11	99	99	99	99	99	99	99
	4 ^c	24/06/96	na	12	472	97	97	97	97	97	97
	5 ^c	26/06/96	1.5 wks	6	221	43	44	44	44	44	44
	6 ^b	29/06/96	na	7	70	70	70	70	70	70	70
	7 ^b	15/08/96	12 wks ^d	6	411	363	78	52	78	53	53
	8 ^b	14/09/96	23 wks	6	379	378	379	0	379	0	0
	9 ^b	15/10/96	18 wks	9	513	511	511	60	511	59	59
	10 ^b	24/11/96	20 wks	16	813	812	812	78	812	77	77
	11 ^b	11/12/96	na	8	373	373	373	29	373	29	29

^a dryland pound

^b tidal pound

^c tank house

^d after \pm 5 weeks holding time (i.e., 23/09/96), a sampling was carried out where 64 lobsters were sampled

na = not available

^e Carapace length, ^f Total haemolymph protein

^g *A. viridans* is the causative agent of gaffkemia

In Lobster Fishing Areas (LFA) where canner-sized lobsters were also landed, only market-sized lobsters were selected for this study. Every lobster from selected crates was individually identified using 25.5 cm long pre-numbered plastic tags, with a pull-tight seal (Ketchum Manufacturing Incorporated, Ottawa, Ontario) placed around the knuckle, proximal to the claw.

2.3.4 Physical assessment

The physical assessment included measurements of body weight (recorded in kilograms), carapace length (CL: measured from the back end of the eye socket to the caudal extremity of the dorsal carapace, and recorded in millimetres), sex, and overall physical condition. The physical condition index was a combination of a subjective assessment of the liveliness of the lobsters and their physical appearance, and was recorded as either normal or downgraded. A lobster was classified as downgraded if it met at least one of the following conditions: dead, weak, missing claw(s), missing leg(s), damaged claw(s), damaged leg(s), missing antenna(e), broken rostrum, damaged body or tail, damaged antenna(e), or any other open wound(s). For details of numbers of lobsters sexed, weighed, measured and assessed for physical condition index in each group, see Table I.

2.3.5 Haemolymph sampling

Haemolymph sampling included the following observations: total

haemolymph protein (THP) and *A. viridans* culture for further identification. For the haemolymph sampling procedure, see Appendix B.

2.3.6 Statistical analysis

Data were entered into a computer spreadsheet (Quattro® Pro version 7, Corel Corporation Limited, Ottawa, Ontario, Canada, 1996). The dataset was transferred into the statistical software STATA™ version 5.0 (Stata Corporation, College Station, Texas, USA, 1996) for further analysis. A random sample of 120 lobster records was examined and manually checked for errors against the original data sheets. Error checking for outliers was conducted by examining descriptive statistics, including means, medians, standard deviations, and minima and maxima for each continuous variable.

The null hypotheses tested were for no differences in various parameters across groups defined by:

- fishing boats or sampling groups,
- time interval (before, during or after impoundment), and
- gender.

Statistical techniques used to evaluate the unconditional associations included Chi-square testing for proportions (Chi-square), one way analysis of

variance with a Bonferroni adjustment for multiple comparisons (ANOVA), paired t-tests for repeated measurements on the same lobsters (paired T) and simple T-tests for comparing group means and assuming unequal variance between groups (T-tests). The parameters considered as dependent variables and the statistical tests used for each evaluation were as follows:

-For fishing boats and sampling groups:

- lobster sex ratio (Chi-square),
- lobster body weight (ANOVA),
- lobster carapace length (ANOVA),
- lobster overall physical condition index (Chi-square),
- lobster THP (ANOVA), and
- *A. viridans* prevalence (Chi-square).

- For time intervals (before versus during, during versus after, and before versus after impoundment):

- lobster body weight (paired T)
- lobster overall physical condition index (paired T),
- lobster THP (paired T), and
- *A. viridans* prevalence (paired T).

For all analyses, differences were considered significant when $P \leq 0.05$.

2.4 Results

2.4.1 Before impoundment

2.4.1.1 Sex ratio

Sex ratios varied from 75.0% males and 25.0% females in one sampling group to 34.0% males and 66.0% females in another sampling group (see Table II). The overall average ratio was 51.8% males and 48.2% females. For details of statistically significant differences in sex ratios among sampling groups, see Appendix C.

Within each sampling group, fishing boat did not affect sex ratio, with the exception of one boat within the group with the highest proportion of males. In that particular sampling group, one boat landed 40% male lobsters, while the overall male proportion for the entire group was 75%.

Gender significantly affected the distribution of lobster body weight, carapace length, THP, and physical condition index. Male lobsters had significantly higher THP levels than female lobsters in two groups. When the gender distribution was assessed for carapace lengths, significant differences were observed in 3 different groups, in which male lobsters had longer carapaces than female lobsters. Male lobsters from these groups also had significantly greater body weight than female lobsters. Finally, when the proportion of male versus female lobsters with normal physical condition was assessed, significant differences were found in two groups. One group had a higher proportion of normal female lobsters, while more normal males were seen in the other group.

Table II. Mean weight and length of tagged lobsters, total haemolymph protein (THP) and the proportion of normal lobsters, the sex ratio, and the prevalence of *Aerococcus viridans* measured before impoundment. Standard deviation in parentheses.

SAMPLING GROUP	GENDER (% male)	LENGTH (mm)	WEIGHT (kg)	PHYSICAL CONDITION (% normal)	THP (g/L)	AEROCOCCUS VIRIDANS	
						PREVALENCE	95% C. I.
1	75.0%	99.2 (15.2)	nd	84.8 (36.1)	99.7 (17.4)	nd	nd
2	43.9%	94.1 (10.7)	0.64 (0.31)	96.3 (18.9)	60.6 (17.5)	na	na
3	70.1%	99.8 (15.3)	0.78 (0.57)	90.9 (28.9)	100.6 (21.3)	na	na
4	34.0%	91.1 (11.5)	0.66 (0.26)	88.7 (31.9)	63.9 (16.2)	nd	nd
5	46.5%	103.2 (18.4)	1.05 (0.71)	84.1 (36.9)	71.8 (17.3)	0%	0% - 8.0%*
6	42.9%	97.2 (14.5)	0.80 (0.39)	84.3 (36.7)	87.1 (18.0)	na	na
7	52.4%	90.5 (6.1)	0.61 (0.14)	68.0 (46.9)	18.1 (4.5)	0%	0% - 6.9%*
8	50.0%	nd	nd	80.0 (40.1)	nd	nd	nd
9	50.8%	90.4 (5.1)	0.60 (0.09)	79.3 (40.6)	37.9 (12.2)	0%	0% - 6.1%*
10	50.5%	98.6 (13.9)	0.81 (0.36)	91.1 (28.4)	64.7 (18.4)	7.8%	2.9% - 16.2%
11	54.4%	100.4 (11.9)	0.88 (0.38)	81.2 (39.1)	78.8 (16.7)	24.1%	10.3% - 43.6%
AVERAGE	51.8%	96.4 (13.6)	0.76 (0.36)	84.7 (36.0)	71.0 (29.6)	2.4%	1.3% - 4.1%

* one-sided, 97.5% confidence interval

na = not available (lost data)

nd = not done

For the specific n values, refer to Table I.

2.4.1.2 Body weight and carapace length

The overall average body weight was 0.76 kg and the average carapace length (CL) was 96.4 mm (ranging from 0.60 kg to 1.05 kg and 90.4 mm to 103.2 mm respectively). For details of statistically significant differences in both weight and CL, see Appendices D and E.

A significant difference in CL was only found in one group between two boats (85.3 mm vs. 93.0 mm). Statistically significant differences in mean lobster weight among fishing boats were found within three sampling groups.

2.4.1.3 Overall physical condition index

The proportion of freshly caught lobsters with normal physical condition index ranged from 79.3% to 96.3% from one group to another (see Table II). The overall average for the proportion of normal condition was 87.3%. For details of statistically significant differences in these proportions, see Appendix F.

Significant differences among fishing boats in the physical condition index of the lobsters landed were observed within three groups. For example, one sampling group had the most statistically significant internal variation with less than 45% normal lobsters in one boat compared to 100% in another boat. In a second group, less than 66% of the lobsters from one boat were scored as normal while this proportion was more than 91% for another boat. The proportion of normal lobsters in two different boats from one other group were lowest at 76% and 80%, while four other boats landed 100% of normal lobsters.

2.4.1.4 Total haemolymph protein

THP levels varied from 18.1 g/L to 100.6 g/L from one sampling group to another (see Table II). The overall average level was 71.0 g/L. For details of statistically significant differences in THP levels among sampling groups, see Appendix G.

Statistically significant THP differences among fishing boats were also found within two lobster groups. For example, in one group the mean THP for lobsters from one particular boat was 77.7 g/L, which was significantly lower than the mean THP from another boat, at 108.1 g/L. In a second group, lobsters from one boat had a mean THP (77.0 g/L) significantly higher than lobsters from all other boats, except one (64.9 g/L).

A statistically significant difference in THP levels was observed between lobsters with normal physical condition index and lobsters with downgraded index. Downgraded lobsters had THP values lower than normal lobsters, with a mean difference of 10.3 g/L ($P<0.001$).

2.4.1.5 *Aerococcus viridans*

Only two groups had prevalence of *A. viridans* greater than zero before the impoundment period (see Table II). Lobsters from one sampling group had prevalence of *A. viridans* of 7.8%, (95% confidence interval = 2.9% - 16.2%) while the prevalence was 24.1% for another group (95% CI = 10.3% - 43.5%). Although no sampled lobsters were positive for *A. viridans* in three other groups,

the maximum limits for the 97.5% confidence interval were 8.0%, 6.9% and 6.1%. For details of statistically significant differences in *A. viridans* prevalence among lobster sampling groups, see Appendix H.

The overall prevalence of *A. viridans* was 2.4%. Only within one sampling group were there significant differences among fishing boats observed in the prevalence of *A. viridans*. The estimated prevalence was 60% in one boat, which was significantly higher than any other boats except three other boats, with prevalence of 20%, 25% and 25% (see Table III).

2.4.2 During impoundment

In sampling Group 7, lobster assessments were carried out during the holding period, i.e., holding day 38. No significant difference among fishing boats was found for any variables. A significant increase in body weight was found between the beginning of the impoundment period and the time of assessment during the impoundment, with an individual mean weight gain of 22.7 g ($P=0.007$). A significant increase in THP levels (THP) was also seen during impoundment, the average increase being 25.8 g/L ($P=0.006$). The decrease in the proportion of normal lobsters was also found to be significant ($P=0.017$), with a relative decrease from 91.7% (pre-impoundment) to 50.0% (after 38 days of impoundment). Finally, no significant differences were found in the estimated prevalence of *A. viridans* between the assessments before and during holding ($P=0.343$).

Table III. *Aerococcus viridans* prevalence with the corresponding 95% confidence interval by fishing boat among lobster sampling group 10. Significant differences ($P \leq 0.05$) in the prevalence among boats are indicated by different superscripts. Common superscripts indicate no difference.

BOAT	N	PREVALENCE	95% C.I.
1	6	0% ^a	0% - 45.9%*
2	5	20.0% ^{a, c}	0.5% - 71.6%
3	6	0% ^a	0% - 45.9%*
4	7	0% ^a	0% - 40.9%*
5	6	0% ^a	0% - 45.9%*
6	6	0% ^a	0% - 45.9%*
7	6	0% ^a	0% - 45.9%*
8	5	0% ^a	0% - 52.2%*
10	5	60.0% ^{b, c}	14.7% - 94.7%
11	2	0% ^a	0% - 84.2%*
12	4	25.0% ^{a, c}	0.6% - 80.6%
13	6	0% ^a	0% - 45.9%*
14	5	0% ^a	0% - 52.2%*
15	4	0% ^a	0% - 60.2%*
16	4	25.0% ^{a, c}	0.6% - 80.6%

* one-sided, 97.5% confidence interval

nd=not done

No significant difference was found in lobster weight, physical condition or THP between the assessments taken during and after impoundment. However the power of detection was relatively low, with 8.1%, 16.6% and 43.6% for body weight, overall condition, and THP, respectively. A power of 8.1% indicates that if a real difference exists, it will only be detected in 8 % of the time.

2.4.3 After impoundment

Data were collected after impoundment for five groups of lobster, when the pounds were emptied. Only one group was assessed for the physical condition index of the lobsters. Although haemolymph was sampled for estimating the prevalence of *A. viridans* after impoundment for four of these lobster sampling groups, most samples were contaminated during processing, resulting in missing data. In the sampling group with uncontaminated haemolymph, no *A. viridans* was detected in the sample (n=113, one-sided 97.5% CI = 3.2%).

2.4.3.1 Lobster body weight

Significant differences in mean body lobster weight among sampling groups were found after impoundment (see Table IV). Body weights after impoundment ranged from 0.61 kg to 0.84 kg with an average of 0.64 kg. One sampling group had significantly heavier lobsters than any other sampling groups after holding. No significant difference after holding among fishing boats was found within each sampling group (see Appendix I). Significant increase in the

Table IV. Results of repeated measures tests (paired T-tests) on lobster weight, physical condition and total haemolymph protein before and after the impoundment period. Standard deviations are presented in parentheses.

GROUP	TYPE OF FACILITY	STARTING DATE OF IMPOUNDMENT	HOLDING TIME (wk)	WEIGHT (kg)		PHYSICAL CONDITION (% normal)		TOTAL HAEMOLYMPH PROTEIN (g/L)		
				BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	
3	5	tank house	26 Jun 1996	1.5	nd	nd	84.09	90.91	nd	nd
	7	tidal pound	15 Aug 1996	12	0.68	0.62	66.67	59.42	19.37 ^a	38.21 ^b
	8	tidal pound	14 Sep 1996	23	nd	nd	69.23 ^a	12.82 ^b	nd	nd
	9	tidal pound	15 Oct 1996	18	0.59 ^a	0.61 ^b	77.49 ^a	46.07 ^b	35.89 ^a	47.57 ^b
	10	tidal pound	24 Nov 1996	20	0.83 ^a	0.84 ^b	94.64 ^a	46.43 ^b	nd	nd

Significant differences between 'before' and 'after' for each parameter (weight, physical condition and THP) are shown by different superscripts ($P \leq 0.05$)

nd = not done

mean lobster weight after impoundment was observed for one group with a mean increase of 14.1 g ($P<0.001$) and also in another group with an increase averaging 13.2 g ($P=0.001$).

2.4.3.2 Overall physical condition index

Significant differences after holding among lobster groups in the mean proportion of lobsters with normal physical appearance were found among every group except between two different sampling groups ($P>0.999$) (Appendix J). Within one of these two groups, significant differences were observed among fishing boats. Lobsters which were landed from one boat had a significantly higher proportion of normal physical condition index than when landed from any other boats with 38.5% compared to 0%-20% for the other boats. No other significant differences among boats were observed within groups of lobsters supplied to each pound.

Table IV also shows the mean proportion of lobsters with normal physical condition index for Groups 5, 7, 8, 9 and 10 before and after impoundment. A significant decrease of 56.4% in the proportion of normal lobsters after holding was observed in one group ($P<0.001$), a significant decrease of 31.4% was found in another group ($P<0.001$), and a significant decrease of 48.2% was observed in a third group ($P<0.001$). Significant differences were absent in the other two groups (Figure 1).

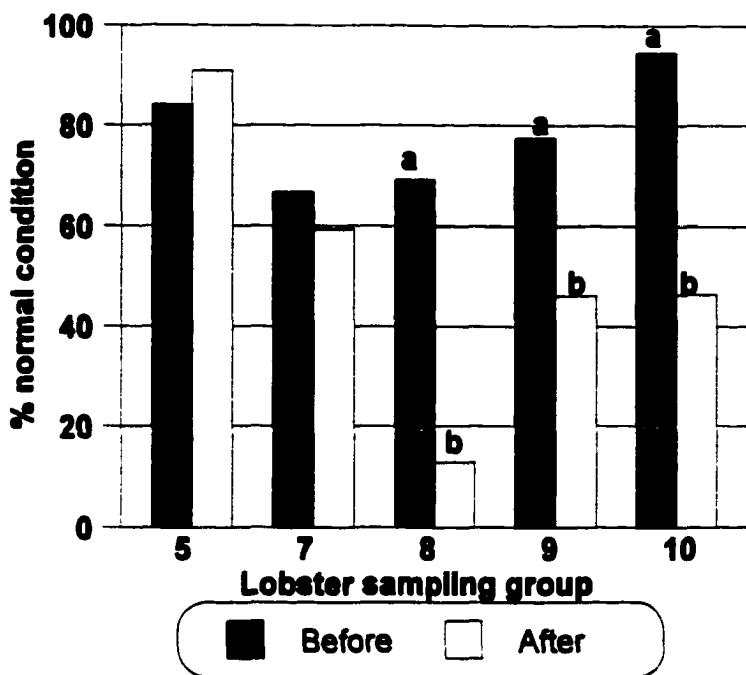


Figure 1. Proportion of lobsters with normal physical condition before and after impoundment for sampling groups 5, 7, 8, 9 and 10. Significant differences within each group are shown by the different superscripts ($n_5=44$, $n_7=69$, $n_8=117$, $n_9=191$, and $n_{10}=56$). The overall average before impoundment was 76.1% and after impoundment was 43.6%, a significant decrease of 32.5% ($T=13.08$, $df=481$, $P<0.001$)

2.4.3.3 Total haemolymph protein

Although no significant differences after holding were found among fishing boats within each holding facility, statistically significant differences in total THP levels were seen among the sampling groups themselves. THP levels ranged from 40.1 g/L to 54.8 g/L with a mean of 43.2 g/L for all groups together. For details of statistically significant differences in THP levels post-holding among lobster sampling groups, see Appendix K.

Significant increases in THP levels after impoundment were also observed in one group with an increase of 18.8 g/L ($P<0.001$), and in another group with an increase of 11.7 g/L ($P=0.007$).

2.4.3.4 Mortality rates

The recovery of tagged lobsters after impoundment varied from 39.1% to 100% with an average of 79.8% (see Table V). Because approximately half of the lobsters of one particular group were marketed without the investigators being notified, the recovery proportion was less than 40%. Statistically significant differences in the observed mortality rates were present among some sampling groups (see Table V).

No significant differences were found among fishing boats within sampling groups for lobster mortality rates over the impoundment. In one sampling group, marginal differences ($P<0.100$) were observed. Lobsters from one boat had a higher mortality rate calculated at 0.54% per week, when the overall mortality rate for this group was estimated at 0.15% per week of holding.

Table V. Percent of tagged lobsters recovered, shrinkage (total volume difference after impoundment) and mortality rates (per week) of tagged lobsters after the impoundment period, in lobster sampling Groups 5, 7, 8, 9 and 10.

GROUP	TYPE OF FACILITY	STARTING DATE OF IMPOUNDMENT	HOLDING TIME (wk)	RECOVERY (%)	SHRINK RATE ¹		MORTALITY RATE ¹	
					ALL	OBSERVED	ALL	OBSERVED
5	tank house	26 Jun 1996	1.5	100%	na	na	0/221 (0%/wk)	0/221 ^a (0%/wk)
7	tidal pound	15 Aug 1996	12	91.7%	1.58 kg/wk (0.64%/wk)	0.13 kg/wk (0.06%/wk)	37/411 (0.75%/wk)	3/377 ^a (0.07%/wk)
8	tidal pound	14 Sep 1996	23	39.1%	nd	nd	268/379 (3.08%/wk)	37/148 ^b (1.09%/wk)
9	tidal pound	15 Oct 1996	18	76.6%	5.55 kg/wk (1.82%/wk)	1.58 kg/wk (0.67%/wk)	173/513 (1.87%/wk)	53/393 ^c (0.75%/wk)
10	tidal pound	24 Nov 1996	20	89.3%	3.25 kg/wk (0.49%/wk)	+0.27 kg/wk (+0.05%/wk)	108/813 (0.66%/wk)	21/726 ^a (0.15%/wk)

¹ The observed rates were calculated only from the lobsters on which data were collected after holding, while the rates for 'all' assumed that all missing data were from dead lobsters with lost tags. Significant differences in the observed mortality rates are shown by different superscripts. Common superscripts indicates no difference (P>0.05)

na = not available

nd = not done

2.4.4 Shrinkage components

Only three groups were included in the estimation of shrinkage, because of lost data from the other groups. The estimated mortality rate was greater than the observed shrinkage rate with an increase in individual lobster weight after the holding period in every group (see Table V). For example, in one group, the total shrinkage rate was estimated at 1.59 g/week or 0.64%/week, while the total estimated mortality rate was 37 lobsters out of 411, or 0.75%/week. Two shrinkage and mortality rates were calculated for each group; one assuming that all missing lobsters were dead and the tags were lost, and the other assuming that recovered lobsters had rates equal to dead lobsters (see Table V).

2.5 Discussion

2.5.1 Lobster condition before impoundment

Significant differences were observed for every index used to describe lobster condition and health among the different sampling groups, and therefore, among different fishing wharves. Furthermore, significant differences in the same indices were also present among fishing boats from the same wharves. A certain degree of random variation in the data collected was expected among the different sampled populations. Physical assessment and condition index, and THP were measured on lobsters from different geographic regions, at different times of the year, with different water temperatures, and most probably where different sources of food and prey were available. All these factors affect plasma protein, molting cycle (Ennis, 1973; Paterson and Spanoghe, 1997), and possibly

overall health status. Unless ill or weak lobsters, ovigerous females (i.e., with eggs externally attached to their tails), or lobsters in different molting stages are attracted by different bait and perhaps basic trap design, the physiological parameters of those lobsters entering the traps should be similar. Variation in the quality of lobsters landed from one particular population may reflect a change of these indices on board fishing boats, and therefore could be linked to different fishing practices. Statistically significant differences in sex ratio, lobster weight, carapace length, and prevalence of *A. viridans*, in freshly caught lobsters from different fishing wharves were also expected. This variation was obvious in this study. However, some significant differences in sex ratio, lobster size and *A. viridans* prevalence were observed within some sampling groups. These variations were unexpected since lobster boats from one particular wharf should fish lobsters from a common population of wild lobsters; most fishers usually setting their traps in the same fishing grounds. No biological explanation is available for these findings.

Canadian regulations stipulate that ovigerous females must be returned to the water (Miller, 1995), thus increasing the likelihood of landing a lower proportion of females than males. Gender was found to be related to some parameters, in some sampling groups. When a significant difference in lobster size according to sex was found, male lobsters tended to be larger. Cobb (1995) suggested that male lobsters should dominate the sex ratio in heavier populations, since they tend to grow to greater sizes, which paralleled the findings of this study. Within two sampling groups, male lobsters also had

significantly higher levels of THP. Reports of gender differences in the values of THP in crustacean are inconsistent, with most studies reporting little or no gender effect (Mackenzie *et al.*, 1997a; Chen and Chia, 1997; Jussila *et al.*, 1997). No significant relationship could be identified between lobster size (body weight or carapace length) and THP. Mackenzie *et al.* (1997a) reported THP values for American lobster averaging 67.8 g/L, which is well within the THP values of this study, with 95% of the population included between 11.9 g/L and 130.1 g/L, and a mean of 71.0 g/L.

The only physiological index used in this study was THP. Paterson and Spanoghe (1997) suggested that haemolymph parameters such as THP could be used as stress indicators in marine decapods. In this study, downgraded lobsters showed significantly lower THP levels, indicating that THP levels could be used as reliable predictors of lobster overall physical condition and probably as lobster stress indicators.

There was a greater proportion of lobsters with an overall physical condition scored as 'normal' when the assessments were carried out directly at the wharves, compared to assessments after the shipments arrived at the holding facilities. This difference in physical condition index was likely attributable to the extra handling and stress involved with the additional manipulations during transportation between wharves and lobster pounds. Perhaps different fishing practices on boats, or different weather conditions during fishing could also have inflicted additional stress on the lobster that was then observed at the processing plants. For crustaceans, certain procedures

such as storage and out-of-the-water-transportation, can be significant stressors, and may result in weaker individuals (Paterson and Spanoghe, 1997).

Assessing the physical condition of lobsters directly at the fishing wharf, as they were landed by the fishers, short-circuited the transportation step, and thus probably documented a 'better' product. In the western rock lobster (*Panulirus cygnus*) industry, significant economic losses are linked to the physical stress induced by lobster harvesting and handling (Paterson and Spanoghe, 1997), and those factors could cause some of the productivity losses seen in the Canadian lobster fishery.

The volume of lobsters landed per boat was not recorded, but would most likely have shown significant variation among boats. Romaire (1995) indicated that numerous factors can affect the trap catch in commercial crawfish (*Procambarus clarkii*) aquaculture, including trap design, water quality, bait, weather, and population density. Varying behavioural responses of decapods to different types of feed, natural or manufactured, are well documented (Kreider and Watts, 1998) and consequently, the type of bait used and the basic design of the traps, should also have varying success. Factors that affect the trap catch (i.e., water quality, types of bait, weather and population density) could also be associated with the health of lobsters being captured, and therefore partly explain some of the variations in THP levels and physical condition seen among fishing wharves, and fishing boats. Other fishing practices should also be investigated, to further explain fishing boat variation in both the quantity and quality of the landed product.

2.5.2 Impoundment consequence

When it was possible to assess lobsters both before and after impoundment, significant differences in health indices were observed. Although lobsters from one group did not show a significant weight increase or a significant change in the proportion of normal lobsters, there was a significant increase in THP levels, indicating physiological changes probably due to the molting cycle. One other sampling group did not show a significant change in the proportion of normal lobsters after the holding period. However, these two groups with no variation in the physical condition index had the shortest holding periods (1.5 and 12 weeks respectively). Perhaps this holding time was of an insufficient duration to observe significant changes in the body weights of the lobsters, or in their physical condition.

When a significant change in the average lobster body weight was observed after impoundment, it was always a weight increase. In the early part of the fall, the Canadian lobster industry will typically purchase recently-molted lobsters from Maine, USA, and hold these animals for various periods, to harden their shells and to substantially increase their body weight. Although the mean individual change in body weight during this study only represented an increase of 4.55 g to 13.64 g, any increase would generate substantial economic benefit. The weight gain observed in this study translates into 0.7% to 2.3% weight, or \$9,240 to \$30,360 for a holding facility with a capacity of 100,000 kg, excluding mortalities, and assuming a fixed price of \$13.20/kg for every harvested lobster (i.e., no difference in price for normal versus downgraded lobsters).

The significant decreases in overall condition seen after impoundment were expected, as lobsters are aggressive and cannibalistic, especially when held in close confinement. Due to agonistic behavioural interaction, lobsters kept in confined areas will inflict physical injuries to each other (Aiken and Waddy, 1995).

The physiological parameter THP probably reflects lobster health only to a certain degree. Among many factors, THP is affected by the molting cycle. Since molting is regulated in part by water temperature, a variation in THP was expected during this study as water temperature increased. Immediately following the completion of the ecdysis, lobsters ingest and absorb substantial volumes of seawater to expand to volumes often 50% greater than their body volume prior to ecdysis (Aiken and Waddy, 1992). This considerable ingestion of water results in haemolymph dilution with total haemolymph volume rising from 30% of the lobster's wet weight during molt stage D to 55% immediately after ecdysis (Martin and Hose, 1995), which may explain lower THP levels observed. The lowest THP levels were observed with the newly-molted Maine lobsters assessed in the early part of fall. High levels of feeding activity could be necessary to enable lobsters to recover from the immediate postmolt low condition, suggested by varying serum protein in field-captured lobsters (Ennis, 1973). Therefore, THP should gradually return to pre-ecdysis levels following stage B of the molt cycle, which was indeed observed as a significant increase in THP levels after impoundment.

2.5.3 Shrinkage

An accurate assessment of the shrinkage rates within each pound was not achievable during this study. The lobster industry is characterized by unpredictable marketing decisions. This prevented the investigators from performing many of the lobster physical condition assessments and recovering data from the final point of handling (i.e., when the pounds were emptied). However, in one group, almost 92% of the tagged lobsters were retrieved when the pound was harvested. The remaining 8% either lost their tags, died, were live lobsters with retained tags but which were sent to processing or marketing without being noticed, or some combination of these factors. If a tagged lobster died during impoundment, it most likely was cannibalized by other lobsters or simply degenerated, releasing the tag into the water. The physical nature of a tidal pound enabled the floating tags to be retrieved by employees, but often the tags either washed ashore or were lost with the tides. Therefore, the minimum true mortality rate was the mortality rate observed among all recovered tagged lobsters and the maximum true mortality rate assumes that all missing tags represent dead lobsters. Due to the average lobster weight increase during impoundment, the shrinkage rate, as perceived by the pound operators, consisted of a combined effect of mortality rates and weight gain in survivors, which can be summarized by the total biomass purchased minus the total biomass sold over the holding period. For example, a shrink rate of 0.06% per week could represent 0.08 % mortalities per week combined with a weight gain of 0.02% per week. However, this calculation only includes live-weight gain or

loss and the mortalities, and an error term should probably be included to account for escapees and other uncontrollable factors. Since the industry traditionally works by total weight and not by the total number of lobsters, getting a rough estimate of mortality rate can only be achieved via weight averages of lobsters going in and coming out of the pound. For the fall pounding season of 1997, pound operators in Maine and New Brunswick routinely observed shrinkage proportions in tidal pounds to average 12%, and is similar to the observations in this study.

2.6 Conclusions

The primary objectives of this chapter included the assessment of the variation in lobster health among different fishing ports in New Brunswick, Nova Scotia and Prince Edward Island, and among different fishing boats from these ports. Both of these objectives were achieved. Secondary objectives included the quantification of productivity losses during lobster holding and assessment of factors contributing to these losses. Unfortunately, these objectives were difficult to fully achieve because of the substantial amount of missing data following impoundment. Nevertheless, important and valuable information regarding these secondary objectives was obtained.

Significant variation in all parameters examined during the course of this study were seen among the different sampling groups. Significant variation among fishing boats in the overall physical condition index of lobsters landed at common wharves was clearly documented. Although the influence of boat

factors has long been suspected, identification of the boat as an influence on health and productivity is a major advancement. If the environment and weather conditions in which these lobsters were caught are similar among boats fishing from common wharves, it is likely that the fishing practices themselves had substantial impacts on lobster health. Because of the logistics of following tagged lobsters through the handling points in the industry, it was difficult to obtain valid assessments of possible boat factors. The assessment of specific fishing practices should be further investigated.

One of the major limitations of this study was the unpredictable harvesting decisions made by many of the lobster facility operators or managers regarding emptying the pounds. Getting to the facilities in time to collect final data was often impossible for the investigators, thus explaining the low final data recovery proportions seen in many pounds. To get a complete and accurate description of shrinkage, its components, and their economic consequence, further studies should be conducted, that incorporate mortality rates and weight changes under different conditions. Data collection would be enhanced by having the investigators present during the entire impoundment duration, compared to depending on notification from the holding facility employees regarding the expected time of harvest. Having one person dedicated to collecting study information at the lobster pound would allow for the collection of data during the entire impoundment period, thus enabling the investigators to obtain a better measure of weight gain or loss over time, mortalities, variation in physical condition index, and THP levels.

Adding other physiological parameters, such as total haemocyte counts or haemocyte differentials should also yield useful information. Having a health or stress indicator that would not vary with water temperature, season, molting cycle, or reproduction status would be extremely valuable. THP was related to the lobster overall physical condition index, and appears to be a potential stress indicator. Paterson and Spanoghe (1997) suggested a series of different stress indicators for marine decapods, with special reference to the western rock lobster, such as inorganic ions, oxygen and oxygen uptake, metabolites and waste products, hormones and proteins. The use of these indicators at the individual level should be applicable to the Canadian lobster industry, and should be investigated for their potential roles as management tools for a more productive fishery. Having indicators that could be applied at the group level would most likely be more efficient, although less specific, as the only group level indicator presently used by the industry consists of historical data for a particular buyer, wharf or boat.

**3. DESCRIPTIVE STATISTICS OF HANDLING, FISHING PRACTICES,
POST-HARVEST HEALTH STATUS AND TRANSPORT CONDITIONS
IN THE PRINCE EDWARD ISLAND LOBSTER INDUSTRY**

3.1 Introduction

The lobster (*Homarus americanus*) fishery is one of the last stable fisheries in Canada. While Canadian landings reached a record peak in 1991 with more than 48,500 metric tons, they have remained more or less stable since 1992 at about 40,000 metric tons, with an estimated value of almost \$400 million (Can.) in 1997 (Fisheries and Ocean Canada, 1999). In 1996, Prince Edward Island fishers landed a total of 8,068 metric tons (20.4% of total Canadian landings) with a value of more than \$61 million (Can.) (Fisheries and Ocean Canada, 1999). According to the Canadian Atlantic Lobster Promotion Association, about 50% of the Canadian catches are destined for live market, while the other half is delivered to the customers as processed product, i.e., cooked, partially cooked or fast frozen.

To supply the market with live product year-round, the industry keeps live lobsters in captivity for various periods, ranging from a few hours to several months. However, mortalities pre-processing cause tremendous losses. These losses have been estimated by the lobster industry to be in the range of 10-15% (Lobster Health Research Centre, 1999), with an economic value of \$50-75 million (Can.). Consequently, the ability of the industry to maximize economic returns is reduced by lack of knowledge of factors contributing to these losses.

Presently there are no cohesive strategies which link all participants, from fishers to consumers, to detect and quantify production inefficiencies. The likelihood of disease outbreaks is usually increased in confinement situations (eg lobster pound), especially when many animals from various sources are kept together, compared to natural environment (Radostits *et al.*, 1994). Infectious diseases should be considered among the possible causes for mortalities during holding. Gaffkemia, caused by *Aerococcus viridans* var. *homari*, is probably one of the most important infectious diseases of impounded lobsters, and it is endemic in stocks of *H. americanus* in North American waters (Alderman, 1996). Gaffkemia is usually high on the list of differential diagnoses when mortalities occur in live holding facilities. Bumper car disease, or ciliate disease, caused by the protozoan scuticociliate *Anophryoides haemophila* (Cawthron *et al.*, 1996), can also reduce lobster survival, especially during winter impoundment.

The lobster fishery is primarily a specialized inshore small boat fishery (Pringle and Burke, 1993). There is consensus in the Canadian lobster industry that important variation in the quality of the product landed by different fishing boats, and among different fishing wharves is present, although no published data are available. Variations in lobster health at time of arrival at processing plants and storage facilities and when lobsters are removed from short-term or long-term holding can be partially attributed to conditions at the time of landing. Therefore, wharf-level factors and boat-level factors directly influence lobster health. To fully understand the total variation of lobster health which is affected by fishing boats, further assessment at the boat level is needed. Paterson &

Spanoghe (1997) suggested that sampling lobsters at various points of handling should yield valid information on stressors causing fatigue, weakness and death.

Following and assessing lobsters through the different handling points enabled the investigators to obtain a precise estimate of variation in lobster health, and identifying critical control points, i.e., factors with significant impacts on lobster productivity. Identification of the frequency of losses and correlation to specific fishing or handling practices could contribute to better management. Subsequent reduction of losses, even by minimal amounts, could provide significant increases in economic returns to the lobster industry. Fishers, buyers, pound operators, processors and exporters might change their handling methods if provided with results that demonstrate where during lobster post-harvest handling the product is losing value.

3.1.1 Objectives

The primary objective of this study was to quantify post-harvest losses in the lobster industry by tagging lobsters directly on the boats, assessing their health, and following them through the different handling points of the Atlantic Canadian industry. The second objective was to describe handling and fishing practices, and lobster transportation conditions in Prince Edward Island during the spring and fall fishing seasons. The final objective was to identify where post-harvest losses occur (i.e., fishing boats, wharves, during transport, holding facilities or processing plants), and to compare the two fishing seasons of Prince Edward Island.

3.2. Materials and methods

Several lobster health indices were monitored on fishing boats during the 1997 May-June fishing season in Lobster Fishing Areas (LFA) 24 and 26. Lobster health was also monitored at wharves and processing plants where the lobsters were handled. Sampling was repeated during the early part of the 1997 August-October fishing season in LFA 25.

The different regions of the Prince Edward Island from which lobsters and fishing boats were assessed and sampled were categorised into four geographic zones referred to as Zones A to D (Figure 1). Zone A corresponded to LFA 24 and comprised the waters between North Cape and East Point, on the north shore, while Zone B referred to the region located between East Point and Beach Point, on the east end of Prince Edward Island which corresponded to LFA 24b. Zone C corresponded to LFA 24a, and included the waters found between Beach Point and Borden, on the south shore. Finally, the region between Borden and North Cape, on the south west end of the province was named Zone D and represented LFA 25.

The first three zones were sampled during the spring season while the fourth zone represented the fall lobster fishing season. The number of fishing wharves sampled per zone was randomized and the corresponding date of sampling was also randomized via the order in which wharves were sampled, as generated by the random list. A total of two wharves in Zone A, eight in Zone B, two in Zone C and three wharves from Zone D were included in the study.

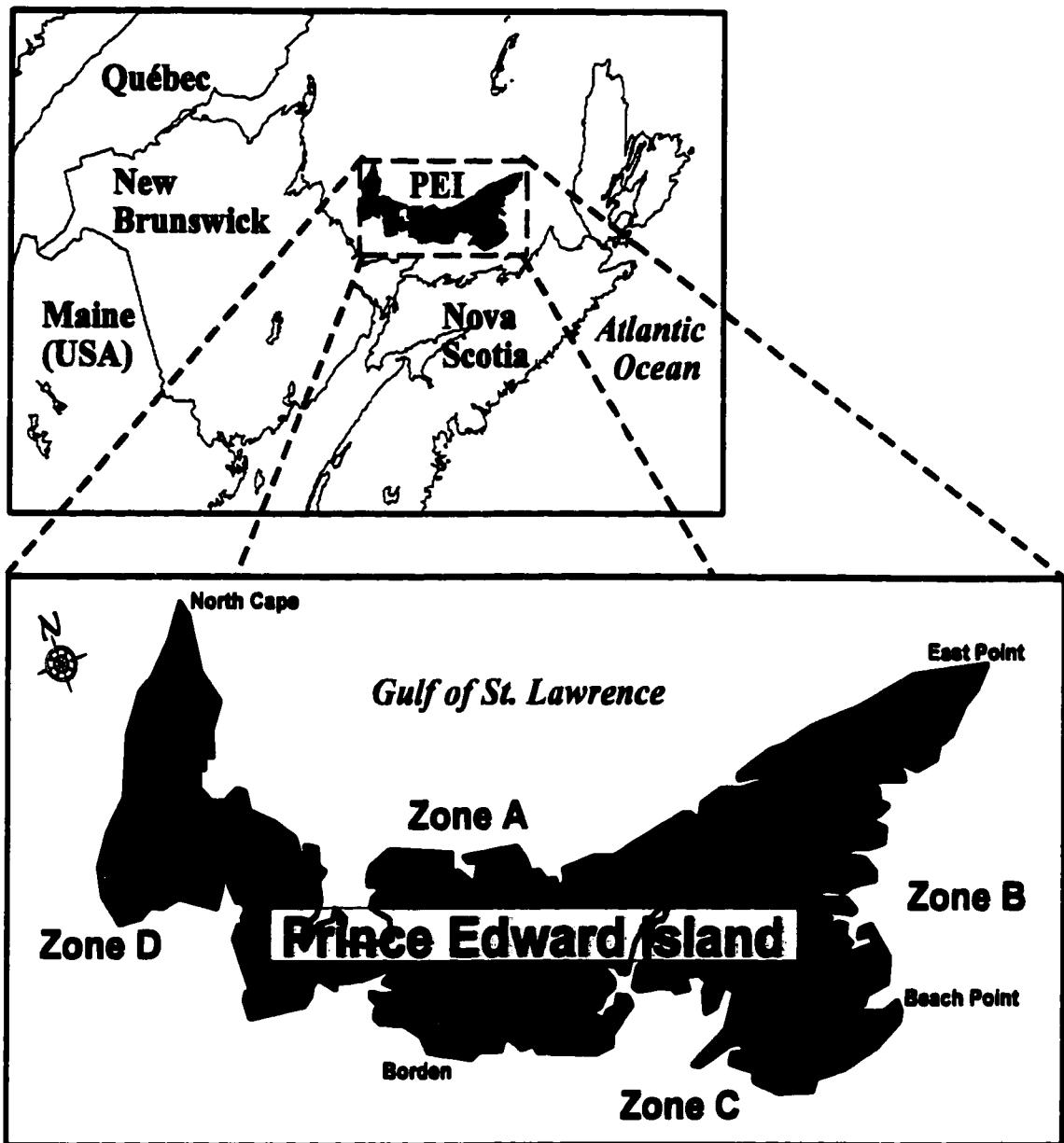


Figure 1. Schematic representation of the four fishing regions sampled from the province of Prince Edward Island, Canada, from May to August, 1997.

3.2.1 Fishing wharf selection

A list of all lobster fishing wharves located in Prince Edward Island was provided by the Department of Fisheries and Oceans Canada (University Avenue, Charlottetown, PE). A random list of all lobster fishing wharves with more than ten boats per port was computer generated (Minitab®, version 10.1, Minitab Incorporated, State College, Pennsylvania, USA, 1994) and divided into two components, either spring or fall fishing season. On average, two wharves were sampled per week, depending on weather and fishers' cooperation, starting with the first wharf on the list for each season.

3.2.2 Fishing boat selection

A brochure describing the project was distributed to fishers at each wharf sampled. Fishers were asked to accommodate an extra person on board to tag and assess lobsters and also assess fishing practices; participation was voluntary. Fishers were selected randomly when more participants than needed agreed to cooperate. When insufficient numbers of fishers were willing to participate, the wharf was not included in the study. Sampling was then conducted at the next fishing wharf on the list.

3.2.3 Lobster selection

Only market-sized lobsters (minimum carapace length of 81 mm) were included in this study. Individual lobsters were identified by placing a 25.5 cm one-piece pre-numbered plastic tag with a pull-tight seal (Ketchum

Manufacturing Inc., Ottawa, ON) around the knuckle, proximal to the claw. A sample size of 50 market-sized lobsters per boat was attempted; historical data obtained from fishers indicated that 50 would be the average number of market-sized lobsters landed per boat. The investigators wanted to include as many market-sized lobsters landed on each study boat, while avoiding the situation where a small number of boats might contribute a disproportionate number of lobsters to the study. Every lobster in the study was given a health assessment (see Section 3.2.4) and, when possible, was reassessed at the holding facility or processing plant. Due to fluctuating landing sizes, fishers did not always catch 50 market-sized lobsters every day. The average landing size of the three previous fishing days (excluding Monday) was used to estimate the proportion of lobsters required to tag 50 lobsters. If one fisher expected less than 50 lobsters, every lobster caught was tagged and assessed. If the investigators had reasons to believe that more than 50 lobsters would be caught, then the randomized systematic sampling interval was adjusted to obtain lobsters throughout the entire fishing day.

3.2.4 Lobster health assessment

3.2.4.1 Physical examination

Carapace length (measured in mm from the caudal end of the eye socket to the caudal extremity of the carapace), sex, shell score (Appendix L), and physical condition index were recorded for every tagged lobster. Lobster physical condition index encompassed assessments of damaged or missing

claws, legs and antennae, damaged body, open wounds, and vigour status (normal, decreased or dead). These assessments were performed on the boats immediately after lobsters were removed from the traps. Body weight, recorded in kilograms (Accu-Weigh DSY-1000, Industrial Scales Ltd., Surrey, BC), shell score, and overall physical condition were also measured for every tagged lobster at fishing ports, whenever possible. Body weight, shell score and overall physical condition were reevaluated on every tagged lobster on arrival at processing plants, whenever possible.

3.2.4.2 Haemolymph sampling

On average, 15 lobsters on each boat were systematically selected for haemolymph sampling, including total haemolymph protein (THP), total haemocyte counts (THC) and *A. viridans*. This sampling protocol is described in Appendix B. Whenever possible, the same sampling procedures were repeated when tagged lobsters were landed at the wharf, after they reached the processing plant, and before final processing or shipping.

3.2.5 Assessment of fishing practices

A three-part questionnaire was used on board each fishing boat from which lobsters were sampled (see Appendix M).

Part A of the questionnaire, *Identification and Boat Specifications*, collected data on the crew size and number of years of experience of the captain or owner of the boat. The total length of the boat was recorded in metres.

Part B of the questionnaire, *Environment*, included information on air and water temperature (°C), and strength (where 0=none and 5=storm), duration (1=less than 2 h and 4=more than 6 h), and description of precipitation (intermittent or continuous). Additionally, this part included information on sunshine (0=none and 4=more than 6 h), and wave conditions (0=none and 4=more than 6 feet). Wind speed was also subjectively scored from 0 to 4 (0=none, 1=light, 2=moderate, 3=strong and 4=storm).

The third part of the questionnaire, C, *Fishing Practices*, collected data on how lobsters were caught and handled on the boats. Bait used in the traps was classified as gaspereaux, mackerel, herring, flatfish, eel pout, redfish, crab and other. Each of these categories was recorded as either fresh, or salted and/or frozen. The use of elastic bands on the claws, possible contact among lobsters, separation and partition of lobsters before grading, the use of a tarp, having the lobsters loose on the deck at any point, and 'packing over' and 'dumping' of the lobster, either by the fishers or the buyers, were also evaluated. Packing lobsters over was defined as completely emptying a crate by taking each lobster one by one and re-packing them into another container. Dumping lobsters was defined as transferring the entire crate or tote into another container all at once.

Holding systems before and after grading were recorded with the following categories: wooden crate (holding capacity of 40-50 kg of lobster), plastic tote (capacity of 25-35 kg), barrel (capacity of more than 100 kg), tray (up to 20 lobsters in individual compartments), PVC tube (one or two lobsters/tube), homemade box (capacity varying from 20 to 150 kg), other, and none. The tank

system used on board was recorded as: none, plastic tub or 'X-Actics™ box' (capacity of holding up to four totes), fibreglass tank (capacity of holding up to four totes), or other. The presence of a lid while fishing and on the way back to the port was ranked from 0 to 3 where 0=no lid and 3=totally on.

Additionally, data were collected on the availability of water in the tank system while fishing and on the way back to shore, with the following six categories: 'none;' 'stagnant' where water was poured on the lobsters but with no flushing; 'flow-through' where water was constantly pumped in the live-tank; 'poured on' when water was poured on the lobsters and immediately flushed out; 'ice' if the lobster were kept on ice; and 'other.' Furthermore, information was recorded on the maximum and minimum time one particular lobster could have been on the boat. The maximum time represented the period from when the first lobster was caught until the boat arrived at the wharf, while the minimum time was the same interval for the last lobster caught. These two variables were ranked from 1 to 6, where 1=less than 2 h and 6=more than 10 h. The overall handling of the lobsters on each boat was graded as either generally 'placing' or 'tossing' the lobster from the traps to the temporary holding units. Finally, the trap setting configuration used by the fishers was documented with four categories: single (one trap per buoy), double (two traps per buoy), multiple (more than two traps on a long line marked by two buoys), or a combination of the previous three methods. The depth (maximal and minimal) at which the traps were set was recorded in metres. All classifications were collected for both market-sized and canner-sized lobsters.

3.2.6 Assessment of transport conditions

The different transportation vehicles and conditions were recorded by the investigators using a questionnaire (Appendix N). Data collected included a general description of the vehicle and whether lobsters were transported in an open truck bed (pick-up truck), in a permanently closed transportation compartment (without refrigeration) or in a refrigerated transportation compartment. The outside temperature was recorded in degrees Celsius, and the weather conditions subjectively described as on the boat. Availability and type of ice during transport was noted as either YES or NO, freshwater or saltwater ice. The use of wooden crates or plastic totes was also recorded. Finally, the time interval between the fishing port and the processing plant, and the total time the shipment spent in the vehicle, was scored from 1 to 6, where 1=less than 1 h and 6=more than 8 h. A seventh category was available for unknown time intervals.

3.2.7 Statistical analysis

All data collected were entered in a computer using spreadsheet software (Quattro® Pro version 7, Corel Corporation Limited, Ottawa, Ontario, Canada, 1996). A random sample of 120 records was examined and manually checked for data entry errors against original data sheets. The dataset was transferred into a statistical software package (STATA™ 5.0, Stata Corporation, College Station, Texas, USA, 1996) for further analysis.

Validation of data was done by obtaining descriptive statistics, including mean, median, standard deviation, frequency distribution and range for each variable, looking for outliers. Continuous variables are defined as quantitative variables that have numerical and measurable values (Fisher and van Belle, 1993). Frequency distributions were also generated for each categorical variable. Categorical variables are qualitative variables with values or categories that are nominal or ordinal (Fisher and van Belle, 1993). These variables were simplified into dichotomous variables if obvious distribution patterns were seen. Dichotomous variables are categorical variables that can have only two values (Fisher and van Belle, 1993).

Analysis included descriptive statistics, binomial probability tests for gender ratios, Chi-square tests for comparisons of proportions, T-tests for comparisons of means in continuous variables, multiple comparisons (analysis of variance) with Bonferroni adjustments for categorical variables, and linear regression for association between continuous variables (see Table I). For all analyses, differences were considered significant when $P \leq 0.05$.

3.3 Results

3.3.1 Demographic data

3.3.1.1 Fishing wharves, boats, and lobsters assessed

The number of tagged and sampled lobsters was limited by the daily catch. In total, 2,191 lobsters from 64 boats were physically examined.

Table I. Statements of the hypotheses according to the statistical tests used. For all analyses, differences among groups were considered significant when $P \leq 0.05$.

STATISTICAL TEST	NULL HYPOTHESIS
BINOMIAL PROBABILITY	no difference in <i>gender</i> ratio overall and within each zone
CHI-SQUARE	no difference between proportions of <i>downgraded lobsters, prevalence of Aerococcus viridans, wound, and gender</i> , according to <i>season</i>
	no difference between proportions of <i>downgraded lobsters, prevalence of Aerococcus viridans, and wound, according to gender</i>
ANOVA (MULTIPLE COMPARISONS, BONFERRONI ADJUSTMENT)	no difference in the distribution of the categories of <i>claws, legs, antennae, body, wound and vigour</i> , according to <i>zone, season, gender</i>
	no difference in the prevalence of <i>Aerococcus viridans</i> according to <i>zone</i>
	no difference in the distribution of the categories of <i>rain, sunshine, wave, wind, trap setting configuration, bait, holding system, live-tank, water availability, lid cover, overall handling, and time spent on board</i> , according to <i>season</i>
	no difference in the distribution of the categories of <i>transport vehicles, travelling & shipping intervals, use of ice, and wind during transport</i> , according to <i>zone, and season</i>
T-TEST	no difference between means of <i>air and water temperature, years of experience, depth of traps, body weight, carapace length, total haemocyte counts, and total haemolymph protein</i> , according to <i>season, and gender</i>
LINEAR REGRESSION	no linear relationship between <i>date and total haemocyte counts, and between date and total haemolymph protein</i>

Table II summaries spring and fall sampling of the total numbers of tagged lobsters that were physically assessed, that were followed at least to entry into a processing plant, and had haemolymph samples taken. A total of 74.5% of the lobsters tagged on boats during the spring season were followed to processing plants compared to 61.1% of lobsters studied in the fall season. Finally, 36.4% of the lobsters examined on the boats in the spring were also bled for haemolymph sampling, while this proportion was 31.8% in the fall.

3.3.1.2 Geography and location of the sampled wharves

Significant differences were found among the fishing zones for the following variables: sex, THP, THC, body weight, carapace length and open wounds.

3.3.1.3 Experience and crew size

The mean number of years of experience (standard deviation) of the captain in the spring and fall samples was 18.2 years (11.0) and 27.1 years (13.0) respectively. A significant difference between the two seasons ($P=0.037$) was observed. The overall mean (standard deviation) was 19.1 years (10.9). There was no significant difference in the distribution of the size of the crew between the spring and fall seasons (see Table III). Overall, 55.4% of the crews consisted of two persons, 41% of the fishing boats had a 3 person crew, while the remaining boats (3.6%) had crews of 4 persons.

Table II. Summary of the numbers of wharves, boats and tagged lobsters assessed during 1997 spring and fall fishing seasons in PEI.

SAMPLING SEASON	WHARVES ASSESSED	BOATS ASSESSED	TAGGED LOBSTERS (%)		
			PHYSICALLY EXAMINED	FOLLOWED-UP TO THE PLANT	HAEMOLYMPH SAMPLED
SPRING	11	53	1,672 (100%)	1,245 (74.5%)	609 (36.4%)
FALL	3	11	519 (100%)	317 (61.1%)	165 (31.8%)
TOTAL	14	64	2,191 (100%)	1,562 (71.3%)	774 (35.3%)

Table III. Distribution frequency (%) for the size of the crew, including the captain, for both the 1997 spring and fall fishing seasons in PEI.

SEASON	CREW SIZE			TOTAL
	2	3	4	
SPRING	26 (57.8%)	18 (40.0%)	1 (2.2%)	45 (100%)
FALL	5 (45.5%)	5 (45.5%)	1 (9.0%)	11 (100%)
OVERALL	31 (55.4%)	23 (41.0%)	2 (3.6%)	56 (100%)

No significant difference between seasons.

3.3.2 Physical examination of lobsters

3.3.2.1 Gender

The overall sex ratio of tagged lobsters showed a significantly higher proportion of female lobsters than male lobsters, 51.97 % vs. 48.03% (n=2,180 and $P=0.011$) (Figure 2). When comparing sex ratio among zones, Zones A and D had a significantly higher number of males and Zone B and C had a significantly higher number of females. The biggest difference in sex ratio was seen in Zone C with 67.1% of the lobsters being female (n=228). Zones A, B and D had 43.2% (n=347), 57.0% (n=1,090) and 40.6% females (n=515), respectively. In every zone, the gender ratio differed significantly from a 50% males 50% females distribution.

3.3.2.2 Size

A marked difference in carapace length and body weight was observed among all zones, except between Zone B and Zone D (Figures 3 a & b). Lobsters landed in Zone C were on average bigger, in both carapace length and body weight, than lobsters landed elsewhere (see Table IV)

3.3.2.3 Overall physical condition index

Most of the significant differences in the condition of the claws, legs, antennae, lobster bodies and the shell were between Zone D and Zones A & B, with Zone D having the highest proportions of normal lobsters (see Tables V-IX).

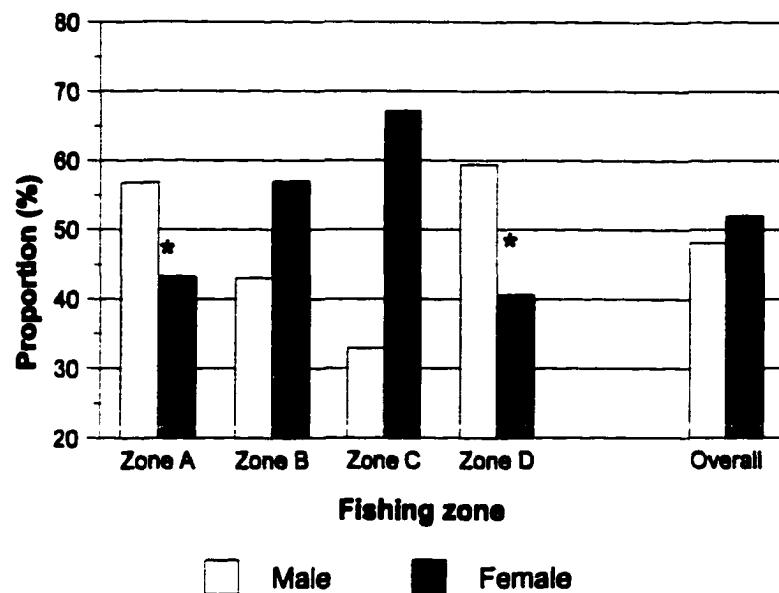
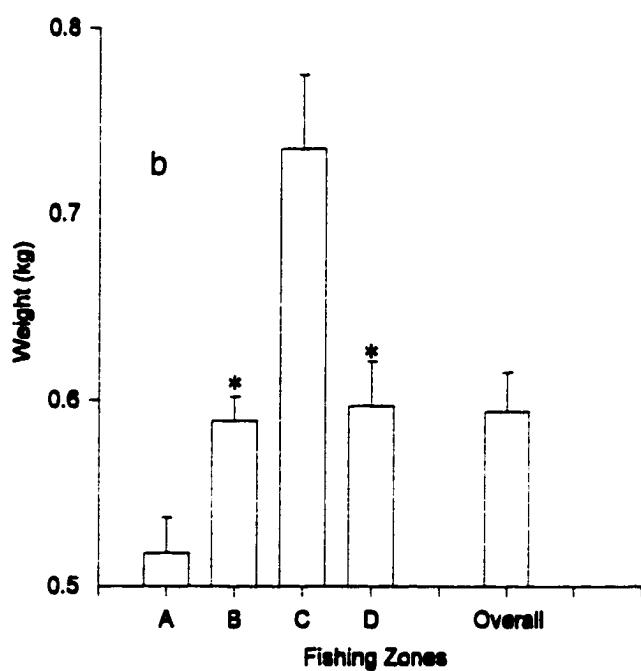
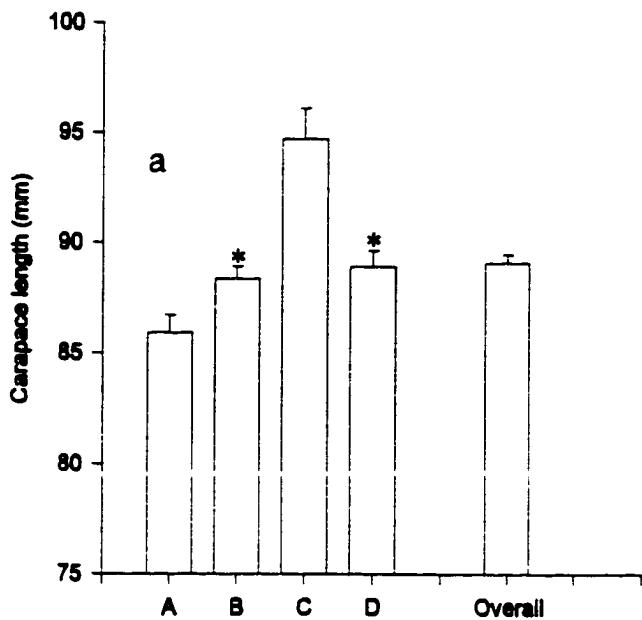


Figure 2. Gender distribution of the tagged lobsters in different sample zones. All zones are different from each other ($P \leq 0.05$) except Zones A and D, as indicated by the asterisk (*). Gender ratios within each zones were all significant ($P \leq 0.05$), as well as the overall ratio.



Figures 3 a & b. Average lobster carapace length (a) and body weight (b) plus the standard error of the mean for each zone as well as the overall average, for all tagged lobsters from the 1997 spring and fall seasons in PEI, measured at the wharves. All zones are different from each other ($P<0.001$) for both weight and length, except for the zones indicated by the asterisk (*).

Table IV. Range, mean and standard deviation of lobster body weight and carapace length, according to source of assessment and fishing zone on PEI during the 1997 sampling.

VARIABLE	SOURCE	ZONE	N	MEAN	RANGE	STANDARD DEVIATION
WEIGHT (kg)	wharf	A	264	0.53	0.26 - 1.82	0.17
		B	821	0.60	0.31 - 2.32	0.23
		C	35	0.76	0.42 - 2.19	0.30
		D	225	0.61	0.35 - 2.18	0.29
	processing plant	A	55	0.63	0.37 - 1.75	0.26
		B	278	0.60	0.33 - 0.66	0.26
		C	nd	nd	nd	nd
		D	50	0.57	0.40 - 1.24	0.16
LENGTH (mm)	boat	A	267	86.32	80 - 142	7.41
		B	929	88.65	77 - 154	9.14
		C	62	95.41	80 - 132	10.61
		D	323	89.30	77 - 145	8.77

nd = not done

Table V. Number of lobsters (percentage) in each physical condition index, at each assessment source, in PEI Zone A in 1997.

PHYSICAL INDEX	ASSESSMENT SOURCE, ZONE A		
	BOAT (n=348)	WHARF (n=293)	PLANT-IN (n=318)
CLAWS			
0 = normal	282 (81.0%)	233 (79.5%)	255 (80.2%)
1 = 1 or 2 damaged	22 (6.3%)	19 (6.5%)	18 (5.7%)
2 = 1 or 2 missing	31 (8.9%)	31 (10.6%)	33 (10.4%)
3 = 1 missing, 1 damaged	13 (3.8%)	10 (3.4%)	12 (3.7%)
LEGS			
0 = all normal	320 (92.0%)	262 (89.4%)	275 (86.5%)
1 = 1 fully or partly missing	22 (6.3%)	16 (5.5%)	21 (6.6%)
2 = 2 fully or partly missing	6 (1.7%)	14 (4.8%)	20 (6.3%)
3 = 3 or + fully or partly missing	0 (0%)	1 (0.3%)	2 (0.6%)
ANTENNAE			
0 = 2 normal	300 (86.2%)	248 (84.6%)	263 (82.7%)
1 = 1 or 2 deformed	8 (2.3%)	6 (2.1%)	7 (2.2%)
2 = 1 or 2 partly missing	1 (0.3%)	2 (0.7%)	2 (0.6%)
3 = 1 or 2 fully missing	36 (10.3%)	32 (10.9%)	40 (12.6%)
4 = 1 fully, 1 partly missing	3 (0.9%)	5 (1.7%)	6 (1.9%)
BODY			
0 = normal	306 (87.9%)	253 (86.4%)	271 (85.2%)
1 = broken rostrum	10 (2.9%)	8 (2.7%)	10 (3.2%)
2 = damaged body	31 (8.9%)	31 (10.6%)	35 (11.0%)
3 = broken rostrum+damaged body	1 (0.3%)	1 (0.3%)	2 (0.6%)
WOUND			
0 = normal	310 (89.1%)	244 (83.3%)	251 (78.9%)
1 = active lesion or open wound	38 (10.9%)	49 (16.7%)	67 (21.1%)
VIGOUR			
0 = normal	346 (99.4%)	290 (99.0%)	312 (98.1%)
1 = loss of vigour	2 (0.6%)	2 (0.7%)	2 (0.6%)
2 = dead	0 (0%)	1 (0.3%)	4 (1.3%)

Table VI. Number of lobsters (percentage) in each physical condition index, at each assessment source, in PEI Zone B in 1997.

PHYSICAL INDEX	ASSESSMENT SOURCE, ZONE B			
	BOAT (n=1,095)	WHARF (n=573)	PLANT-IN (n=854)	PLANT-OUT (n=87)
CLAWS				
0 = normal	953 (87.0%)	490 (85.5%)	728 (80.2%)	72 (82.8%)
1 = 1 or 2 damaged	48 (4.4%)	29 (5.1%)	45 (5.3%)	4 (4.6%)
2 = 1 or 2 missing	85 (7.8%)	48 (8.4%)	72 (8.4%)	11 (12.6%)
3 = 1 missing, 1 damaged	9 (0.8%)	6 (1.0%)	9 (1.1%)	0 (0%)
LEGS				
0 = all normal	1025 (93.6%)	506 (88.3%)	758 (88.8%)	82 (94.2%)
1 = 1 fully or partly missing	50 (4.6%)	37 (6.5%)	45 (5.3%)	2 (2.3%)
2 = 2 fully or partly missing	18 (1.6%)	27 (4.7%)	44 (5.1%)	1 (1.2%)
3 = 3 or + fully or partly missing	2 (0.2%)	3 (0.5%)	7 (0.8%)	2 (2.3%)
ANTENNAE				
0 = 2 normal	954 (87.1%)	494 (86.2%)	709 (83.0%)	59 (67.8%)
1 = 1 or 2 deformed	27 (2.5%)	22 (3.9%)	26 (3.0%)	2 (2.3%)
2 = 1 or 2 partly missing	10 (0.9%)	3 (0.5%)	11 (1.3%)	4 (4.6%)
3 = 1 or 2 fully missing	99 (9.0%)	47 (8.2%)	96 (11.3%)	20 (23.0%)
4 = 1 fully, 1 partly missing	5 (0.5%)	7 (1.2%)	12 (1.4%)	2 (2.3%)
BODY				
0 = normal	1030 (94.1%)	513 (89.5%)	788 (92.3%)	75 (86.2%)
1 = broken rostrum	12 (1.1%)	13 (2.3%)	18 (2.1%)	7 (8.1%)
2 = damaged body	52 (4.7%)	46 (8.0%)	46 (5.4%)	5 (5.7%)
3 = broken rostrum+damaged body	1 (0.1%)	1 (0.2%)	2 (0.2%)	0 (0%)
WOUND				
0 = normal	972 (88.8%)	484 (84.5%)	692 (81.0%)	56 (64.4%)
1 = active lesion or open wound	123 (11.2%)	89 (15.5%)	162 (19.0%)	31 (35.6%)
VIGOUR				
0 = normal	1092 (99.7%)	568 (99.1%)	825 (96.6%)	87 (100%)
1 = loss of vigour	3 (0.3%)	5 (0.9%)	24 (2.8%)	0 (0%)
2 = dead	0 (0%)	0 (0%)	5 (0.6%)	0 (0%)

Table VII. Number of lobsters (percentage) in each physical condition index, at each assessment source, in PEI Zone C in 1997.

PHYSICAL INDEX	ASSESSMENT SOURCE, ZONE C		
	BOAT (n=229)	PLANT-IN (n=73)	PLANT-OUT (n=30)
CLAWS			
0 = normal	191 (83.4%)	59 (80.8%)	24 (80.0%)
1 = 1 or 2 damaged	10 (4.4%)	7 (9.6%)	1 (3.3%)
2 = 1 or 2 missing	23 (10.0%)	6 (8.2%)	4 (13.4%)
3 = 1 missing, 1 damaged	5 (2.2%)	1 (1.4%)	1 (3.3%)
LEGS			
0 = all normal	222 (96.9%)	70 (95.9%)	27 (90.0%)
1 = 1 fully or partly missing	2 (0.9%)	1 (1.4%)	1 (3.3%)
2 = 2 fully or partly missing	4 (1.8%)	2 (2.7%)	2 (6.7%)
3 = 3 or + fully or partly missing	1 (0.4%)	0 (0%)	0 (0%)
ANTENNAE			
0 = 2 normal	197 (86.0%)	59 (80.8%)	26 (86.6%)
1 = 1 or 2 deformed	2 (0.9%)	1 (1.4%)	0 (0%)
2 = 1 or 2 partly missing	0 (0%)	0 (0%)	0 (0%)
3 = 1 or 2 fully missing	26 (11.4%)	11 (15.1%)	2 (6.7%)
4 = 1 fully, 1 partly missing	4 (1.7%)	2 (2.7%)	2 (6.7%)
BODY			
0 = normal	220 (96.1%)	69 (94.5%)	27 (90.0%)
1 = broken rostrum	3 (1.3%)	0 (0%)	0 (0%)
2 = damaged body	6 (2.6%)	4 (5.5%)	3 (10.0%)
3 = broken rostrum+damaged body	0 (0%)	0 (0%)	0 (0%)
WOUND			
0 = normal	188 (82.1%)	63 (86.3%)	27 (90.0%)
1 = active lesion or open wound	41 (17.9%)	10 (13.7%)	3 (10.0%)
VIGOUR			
0 = normal	229 (100%)	73 (100%)	30 (100%)
1 = loss of vigour	0 (0%)	0 (0%)	0 (0%)
2 = dead	0 (0%)	0 (0%)	0 (0%)

Table VIII. Number of lobsters (percentage) in each physical condition index, at each assessment source, in PEI Zone D in 1997.

PHYSICAL INDEX	ASSESSMENT SOURCE, ZONE D			
	BOAT (n=519)	WHARF (n=145)	PLANT-IN (n=317)	PLANT-OUT (n=48)
CLAWS				
0 = normal	483 (93.1%)	136 (93.8%)	286 (90.2%)	48 (100%)
1 = 1 or 2 damaged	13 (2.5%)	3 (2.1%)	6 (1.9%)	0 (0%)
2 = 1 or 2 missing	18 (3.5%)	5 (3.4%)	23 (7.3%)	0 (0%)
3 = 1 missing, 1 damaged	5 (0.9%)	1 (0.7%)	2 (0.6%)	0 (0%)
LEGS				
0 = all normal	509 (98.1%)	142 (97.9%)	307 (96.8%)	48 (100%)
1 = 1 fully or partly missing	4 (0.8%)	2 (1.4%)	3 (1.0%)	0 (0%)
2 = 2 fully or partly missing	5 (0.9%)	1 (0.7%)	7 (2.2%)	0 (0%)
3 = 3 or + fully or partly missing	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)
ANTENNAE				
0 = 2 normal	512 (98.7%)	142 (97.9%)	310 (97.8%)	36 (75%)
1 = 1 or 2 deformed	5 (0.9%)	0 (0%)	5 (1.6%)	0 (0%)
2 = 1 or 2 partly missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
3 = 1 or 2 fully missing	0 (0%)	2 (1.4%)	1 (0.3%)	12 (25%)
4 = 1 fully, 1 partly missing	2 (0.4%)	1 (0.7%)	1 (0.3%)	0 (0%)
BODY				
0 = normal	503 (96.9%)	141 (97.2%)	306 (96.5%)	45 (93.7%)
1 = broken rostrum	4 (0.8%)	1 (0.7%)	1 (0.3%)	2 (4.2%)
2 = damaged body	12 (2.3%)	3 (2.1%)	10 (3.2%)	1 (2.1%)
3 = broken rostrum+damaged body	0 (0%)	0 (0%)	0 (0%)	0 (0%)
WOUND				
0 = normal	495 (94.8%)	135 (93.1%)	292 (92.1%)	35 (72.9%)
1 = active lesion or open wound	27 (5.2%)	10 (6.9%)	25 (7.9%)	13 (27.1%)
VIGOUR				
0 = normal	509 (98.1%)	143 (98.6%)	307 (96.9%)	48 (100%)
1 = loss of vigour	10 (1.9%)	2 (1.4%)	10 (3.1%)	0 (0%)
2 = dead	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table IX. Proportion (%) of lobsters with normal physical indices for each Zone, and at each assessment source during the 1997 sampling in PEI. Significant differences are represented by different superscripts: letters within each row and numbers within each column (one way analysis of variance, with Bonferroni correction, $P<0.05$). No superscript represents no difference. The normal score for the index 'wounds' indicates the absence of wounds.

PHYSICAL INDICES	PROPORTION OF LOBSTERS WITH NORMAL INDICES			
	ZONE A (%)	ZONE B (%)	ZONE C (%)	ZONE D (%)
CLAWS				
boat	81.0 ^a	87.0 ^b	83.4 ^{a, b}	93.1 ^c
wharf	79.5 ^a	85.5 ^a	nd	93.8 ^b
plant-in	80.2 ^a	85.3	80.8	90.2 ^b
plant-out	nd	82.8 ^a	80.0 ^a	100 ^b
LEGS				
boat	92.0 ^a	93.6 ^{a, b, 1}	96.9 ^{b, c}	98.1 ^c
wharf	89.4 ^a	88.3 ^{a, 2}	nd	97.9 ^b
plant-in	86.5 ^a	88.8 ^{a, 2}	95.9	96.9 ^b
plant-out	nd	94.3	90.0	100
ANTENNAE				
boat	86.2 ^a	87.1 ^{a, 1}	86.0 ^a	98.7 ^{b, 1}
wharf	84.6 ^a	86.2 ^{a, 1}	nd	97.9 ^{b, 1}
plant-in	82.7 ^a	83.0 ^{a, 1}	80.8 ^a	97.8 ^{b, 1}
plant-out	nd	67.8 ²	86.6	75.0 ²
BODY				
boat	87.9 ^a	94.1 ^{b, 1}	96.1 ^b	96.9 ^b
wharf	86.4 ^a	89.5 ^{a, 2}	nd	97.2 ^b
plant-in	85.2 ^a	92.3 ^b	94.5 ^b	96.5 ^b
plant-out	nd	86.2	90.0	93.8
WOUND				
boat	89.1 ^{a, 1}	88.8 ^{a, 1}	82.1 ^{b, 1}	94.8 ^{c, 1}
wharf	83.3 ^a	84.5 ^{a, 1, 2}	nd	93.1 ^{b, 1}
plant-in	78.9 ^{a, 2}	81.0 ^{a, 2}	86.3 ¹	92.1 ^{b, 1}
plant-out	nd	64.4 ^{a, 3}	90.0 ^{b, 1}	72.9 ²
VIGOUR				
boat	99.4	99.7 ^{a, 1}	100 ^a	98.1 ^b
wharf	99.0	99.1 ¹	nd	98.6
plant-in	98.1	96.6 ²	100	96.9
plant-out	nd	100	100	100

nd = not done

However, these differences were not always statistically significant, which was the case between Zones D and C for the condition of the legs and bodies, and between Zone D and Zone B for the body condition. Significantly higher proportions of lobsters with open wounds and lobsters with abnormal antennae were seen in Zones B and D, when the lobsters were coming out of the processing plants, compared to boat and wharf assessments.

The total number of lobsters scored for overall physical condition was not always constant at each source of assessment. Depending on the weather, it was not always possible to assess every lobster at the wharves; some buyers allowed the examination of only a certain proportion of the tagged lobsters because of raining conditions or direct sunlight, for example. Some buyers completely refused access to the study lobsters. In some instances, fishers sold part of their daily catch to one buyer, and the other part to another buyer. Therefore, because the investigation team could not be in both places simultaneously, the number of lobsters assessed at the wharves might have been lower than the number assessed on the boats. Finally, in many cases a proportion of the tagged lobsters from one wharf (or fisher) was sold to one processing plant while the remaining lobsters were sold to another plant; assessing the study lobsters at both processing plants was then impossible.

3.3.3 Haemolymph parameters and haematology

THP measured in g/L, and THC, estimated by the number of haemocytes per ml of haemolymph, were measured on approximately 15 lobsters per group

(a group being the batch of tagged lobsters coming from one particular boat), at fisher, buyer and processor points of handling, whenever possible. The prevalence of *A. viridans* in the tagged lobsters was estimated on a subset of lobsters on the boats, and also when lobsters were exiting the processing plants. However, too few lobsters were sampled leaving the processing plants to permit valid assessment of changes in agent prevalence before and after holding.

3.3.3.1 Total haemolymph protein

No assessments were carried out on the wharf for Zone C; lobster groups were sold directly to the processing plant (see Table X). When the source of assessment was the boat, the mean value of THP levels of Zone D was significantly lower than THP levels from any other zone, while no significant differences were found among the other zones. At the wharves, all means were significantly different among all zones. At the processing plants, Zone D had significantly lower THP than Zones A, B and C, while no significant differences were found among the other zones. When Zones A, B and C were assessed together to constitute the spring fishing season, and then compared to Zone D, or fall season, the THP mean values were significantly higher in the spring on boats, at wharves (excluding Zone C) and at processing plants ($P<0.001$) than in the fall. In Zone B, when the THP means were compared among boats, wharves and processing plants, the value of the boats was significantly less than at the wharves (difference=6.7 g/L), while a difference of 6.1 g/L was noted between the wharves and the plants, but not statistically significant ($P=0.056$).

Table X. Range, mean and standard deviation of total haemolymph protein and total haemocyte counts, according to source of assessment and the fishing zone during the 1997 sampling in PEI.

VARIABLE	SOURCE	ZONE	RANGE	MEAN (SD)	N
TOTAL PROTEIN (g/L)	boat	A	16.6 - 108.8	59.02 (19.27)	152
		B	11.9 - 106.9	63.06 (19.08)	408
		C	39.4 - 88.8	59.04 (14.41)	24
		D	17.6 - 110.7	40.7 (16.98)	151
	wharf	A	45.1 - 107.8	62.04 (16.91)	140
		B	36.6 - 112.6	69.72 (16.16)	90
		C	nd	nd	nd
		D	18.5 - 59.4	37.80 (8.74)	45
	processing plant	A	21.4 - 107.8	61.18 (17.23)	122
		B	16.6 - 113.5	63.58 (18.08)	315
		C	27.1 - 104.0	62.07 (17.17)	29
		D	20.4 - 106.9	44.14 (17.96)	104
HAEMOCYTE COUNTS ($\times 10^6$ cells /ml)	boat	A	3 - 51	21.7 (9.95)	140
		B	2 - 58	19.9 (9.66)	333
		C	15 - 46	24.5 (9.23)	25
		D	6 - 50	23.5 (7.96)	148
	wharf	A	13 - 70	30.5 (9.87)	110
		B	12 - 70	33.3 (10.61)	77
		C	nd	nd	nd
		D	6 - 45	27.6 (7.75)	45
	processing plant	A	5 - 65	29.3 (11.35)	101
		B	6 - 94	31.9 (14.48)	269
		C	10 - 62	30.1 (10.62)	27
		D	5 - 66	29.3 (11.49)	104

nd = not done

In the spring, statistically significant differences in THP levels were observed between male and female lobsters on boats, wharves, and arriving and exiting processing plants, with males having consistently higher values than females (see Table XI). However, during the fall sampling, the difference was significant only at wharves and upon arrival at processing plants; males had higher THP values at plants but lower values at the wharves.

Figure 4 illustrates the linear relationship between the date of harvest and the THP levels measured on the boats during the spring season. A positive relationship was observed, with increasing THP as the spring season progressed. No significant relationship was found during the fall season.

3.3.3.2 Circulating haemocytes

Similar to THP, THC were not measured at the wharves in Zone C (see Table X). When lobsters were assessed on boats, a significant difference was only found between Zone B and Zone D ($P<0.001$). During the assessment at the wharf, a significant difference was present between Zones B and D ($P=0.007$). No differences were found at the wharf among the other zones. For the processing plant assessments, no significant differences were found among all zones ($P\geq0.492$). When Zones A, B and C were pooled together, the difference between the spring and the fall seasons is significant on the boats ($P=0.001$) and at the wharves (excluding Zone C, $P=0.015$), with the mean values of THC being lower in the fall, while this difference was not significant at the processing plants ($P=0.208$).

Table XI. Mean values of the total haemolymph protein and total haemocyte counts with standard error, according to the gender of the lobsters, during the 1997 spring and fall seasons in PEI. *P*-values for comparing mean THP and THC values of males vs. females were obtained by t-tests.

PARAMETER	SOURCE	P-VALUE	MALE		FEMALE	
			MEAN (SEM)	N	MEAN (SEM)	N
TOTAL PROTEIN (g/L)						
Spring	boat	0.0001	65.0 (1.27)	331	57.2 (0.86)	248
	wharf	0.0001	70.6 (1.99)	102	60.4 (1.12)	126
	plant in	0.0003	65.3 (1.34)	252	59.5 (0.87)	206
	plant out	0.0020	60.4 (3.70)	35	45.0 (2.67)	22
HAEMOCYTE COUNTS (x 10⁶ cell/ml)						
Spring	boat	0.3151	20.8 (0.63)	284	20.4 (0.58)	212
	wharf	0.0171	33.4 (1.26)	80	30.2 (0.89)	106
	plant in	0.1046	32.0 (1.09)	211	30.3 (0.77)	179
	plant out	0.2020	25.3 (2.82)	23	22.5 (1.47)	19
Fall						
Fall	boat	0.0002	20.6 (0.97)	56	25.3 (0.82)	92
	wharf	0.3985	26.9 (1.52)	6	27.8 (1.32)	39
	plant in	0.0469	31.3 (1.95)	50	27.5 (1.18)	53
	plant out	nd	nd	nd	nd	nd

nd = not done

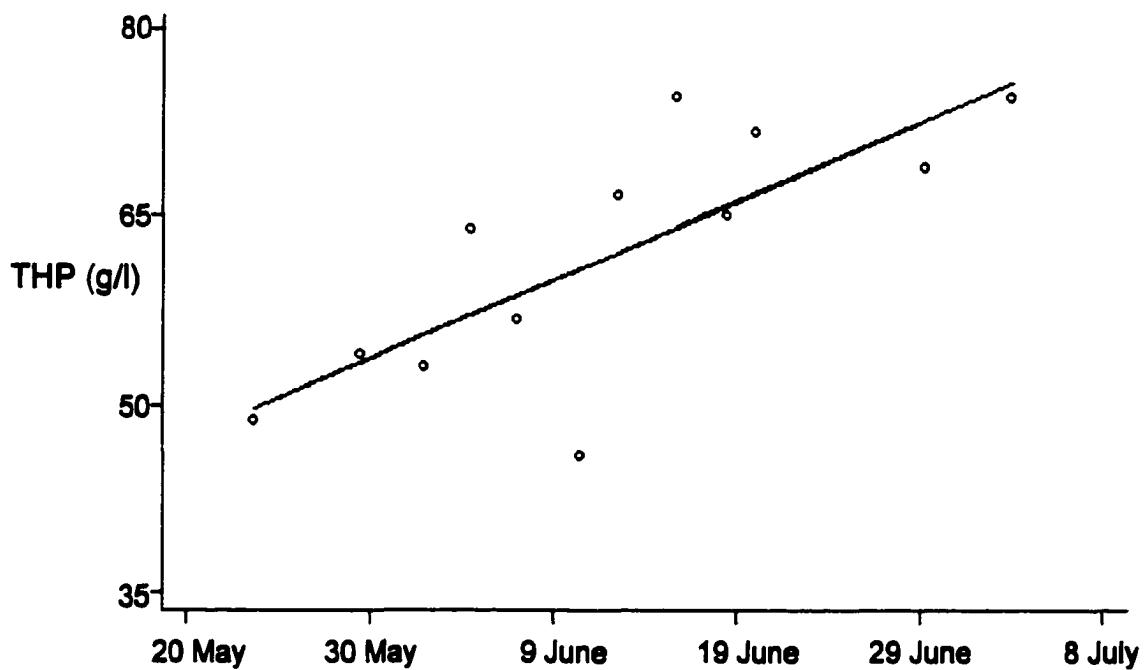


Figure 4. Linear relationship between date of harvest and total haemolymph protein values measured on the boats during the 1997 spring season in PEI ($r^2=0.0671$, $P<0.001$). Each point represents the average value of about 44 lobsters (526 observations divided into 12 groups: 7 groups of 43 observations and 5 groups of 45 observations).

In the spring, although males had higher THC than females on average at every assessment source, a significant difference was observed only at the wharves. During the fall sampling, significant differences between gender were seen on the boats and upon arrival at the processing plants. Males had significantly lower THC than females on the boats, but significantly higher at the plants (see Table XI).

Figure 5 illustrates the linear relationship between the date of harvest and the THC measured on the boats during the spring season. A positive correlation ($P<0.001$) was observed, with increasing THC as the spring season progressed. No significant relationship was found during the fall season. When the means were compared between the different assessment sources, significant increases in THC were found within each zone between boats and wharves ($P\leq0.025$), and between boats and processing plants ($P\leq0.050$). No significant differences in THC were observed between wharves and processing plants ($P\geq0.377$).

3.3.3.3 *Aerococcus viridans*

The results, shown in Figure 6, revealed a higher prevalence of *A. viridans* in the fall season than in the spring season, with 5.73%, 5.50%, 4.00% and 11.33% for Zones A, B, C and D respectively. The overall prevalence in the spring was estimated at 5.50%. No significant differences among any zones were found, but a marginal difference existed between Zone B and Zone D ($P=0.089$). When Zones A, B and C were pooled together, the spring prevalence (5.5%) was significantly lower than the fall prevalence of 11.3% ($P=0.011$).

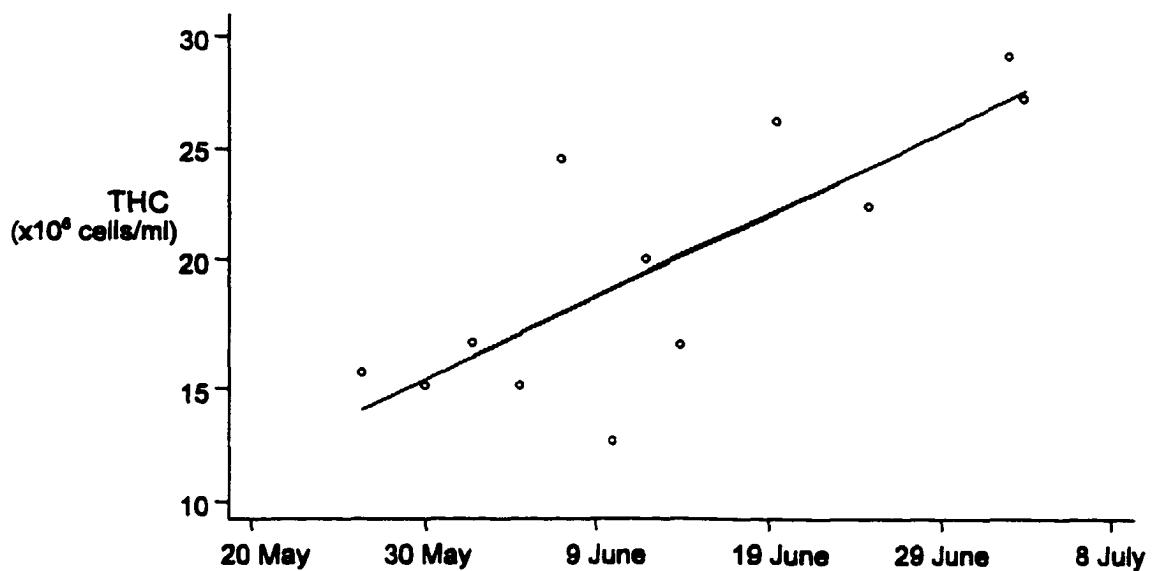


Figure 5. Linear relationship between date of harvest and total haemocyte counts measured on the boats during the 1997 spring season in PEI ($r^2=0.1916$, $P<0.001$). Each point represents the average value of about 37 lobsters (443 observations divided into 12 groups: 11 groups of 37 observations and 1 group of 36 observations).

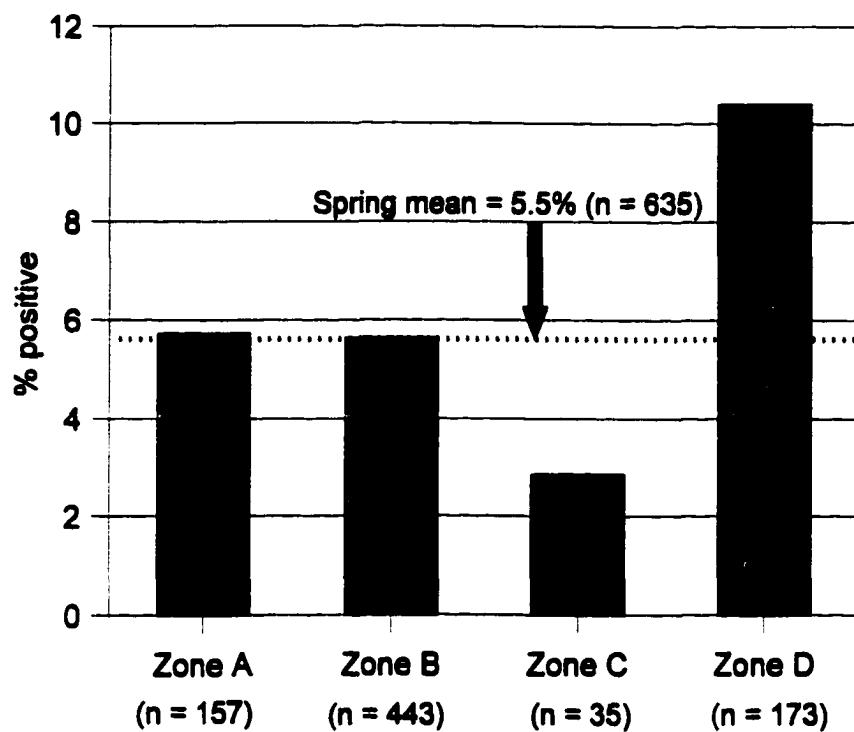


Figure 6. Estimated prevalence of *Aerococcus viridans*, the causative agent of gaffkemia in lobsters, according to the different 1997 fishing zones in PEI. No significant differences were found among any fishing zones.

3.3.4 Environmental factors

The maximum air temperatures ranged from 4 °C to 28 °C, while the minimum air temperatures ranged from 3 °C to 18 °C, with corresponding means (\pm standard deviations) of 16.5 °C (\pm 5.1 °C) and 9.3 °C (\pm 4.2 °C), respectively. The surface water (i.e., less than one metre deep) temperature ranged from 2 °C to 19 °C, with a mean of 9.1 °C and a standard deviation of 4.8 °C.

A significant difference was found in the amount of sunshine during fishing between the spring and fall seasons ($P=0.024$), but not in rainfall. A significant difference was also observed for the wave conditions between the spring and fall seasons ($P=0.012$), while no statistical significant differences were recorded in the wind speed between seasons (see Table XII).

3.3.5 Fishing practices

3.3.5.1 Depth of traps

The overall mean for the maximum depth (\pm SD) was 19.3 metres (\pm 6.7 m) and ranged from 4.5 m to 33.5 m, and the mean minimum depth (\pm SD) was 6.9 m (\pm 5.2 m) and ranged from 1.2 m to 24.5 m. When assessed by season, significant differences were present with both maximum and minimum depth; mean minimum depth was 6.1 m (\pm 4.3 m) in the spring compared to 12.0 m (\pm 7.6 m) in the fall ($P=0.005$). The mean maximum depth was 17.8 m (\pm 6.0 m) in the spring vs. 28.0 m (\pm 3.1 m) in the fall ($P<0.001$).

Table XII. Description and frequency (proportion) of environmental factors monitored on board fishing boats (n = 56) during the 1997 spring and fall seasons in PEI.

ENVIRONMENTAL FACTOR	FISHING SEASON		
	SPRING	FALL	OVERALL
RAIN			
none	33 (73.4%)	7 (63.6%)	40 (61.4%)
light	5 (11.1%)	0 (0%)	5 (8.9%)
mist	6 (13.3%)	2 (18.2%)	8 (14.3%)
moderate	1 (2.2%)	2 (18.2%)	3 (5.4%)
heavy	0 (0%)	0 (0%)	0 (0%)
SUN			
none	11 (24.4%)	2 (18.2%)	13 (23.2%)
<2 h	5 (11.1%)	1 (9.1%)	6 (10.7%)
2-4 h	4 (8.9%)	0 (0%)	4 (7.1%)
4-6 h	20 (44.5%)	2 (18.2%)	22 (23.3%)
>6 h	5 (11.1%)	6 (54.5%)	11 (19.7%)
WAVE			
none	0 (0%)	1 (9.0%)	1 (1.8%)
<2ft	36 (80.0%)	5 (45.5%)	41 (73.2%)
2-4ft	6 (13.3%)	5 (45.5%)	11 (19.6%)
4-6ft	3 (6.7%)	0 (0%)	3 (5.4%)
>6ft	0 (0%)	0 (0%)	0 (0%)
WIND			
none	0 (0%)	1 (9.1%)	1 (1.8%)
light	33 (73.3%)	6 (54.5%)	39 (69.6%)
moderate	11 (24.5%)	4 (36.4%)	15 (26.8%)
strong	0 (0%)	0 (0%)	0 (0%)
storm	1 (2.2%)	0 (0%)	1 (1.8%)

3.3.5.2 Trap setting configuration

Fishers used four different trap setting methods: single (one trap per buoy), double (two traps per buoy), multiple (more than two traps on a long line marked by two buoys), or a combination of the previous three methods. A significant difference ($P<0.001$) in the methods used was found between seasons. Multiple traps per line were used on 95.6% of the boats in the spring, but never used in the fall fishing season (see Table XIII).

3.3.5.3 Bait

The major difference noted between spring and fall seasons was that gaspereaux was not used as bait during the fall, while crushed crab was not used in the spring (see Table XIV). Mackerel was the bait most commonly used during both the spring (41%) and fall seasons (44%) while more herring was used in the fall (27%) compared to the spring (8%).

3.3.5.4 Contact before measuring

The proportion of boats on which it was not possible for the lobsters to have physical contact before being measured and graded was significantly higher in the spring than in the fall ($P<0.001$). During the spring season, lobsters of different sizes were prevented from having mutual contact before grading on 63.6% of the boats (n=53), but only on 18.2% of the boats in the fall (n=11).

Table XIII. Trap setting methods used during the 1997 spring and fall seasons on PEI, with corresponding distribution (proportion) for each category.

TRAP SETTING	FISHING SEASON		
	SPRING	FALL	OVERALL
METHOD			
single	1 (2.2%)	1 (9.1%)	2 (3.6%)
double	0 (0%)	6 (54.5%)	6 (10.7%)
multiple	43 (95.6%)	0 (0%)	43 (76.8%)
combination	1 (2.2%)	4 (36.4%)	5 (8.9%)

Table XIV. Bait used during the 1997 spring and fall fishing seasons on PEI.

BAIT	FREQUENCY OF BAIT USED		
	SPRING	FALL	OVERALL
GASPEREAUX	24.1%	0%	18.1%
MACKEREL	41.4%	44.8%	42.2%
HERRING	8.1%	27.6%	12.9%
FLATFISH	10.3%	3.5%	8.6%
CRUSHED CRAB	0%	17.2%	4.4%
OTHER	16.1%	6.9%	13.8%
TOTAL	100%	100%	100%

3.3.5.5 Holding system and live-tank system

In both fishing seasons, the traditional plastic tote with a capacity of approximately 35 kg, represented the holding unit most used on board fishing boats, whether it was before or after the lobsters were measured and graded (see Table XV).

During the spring, most fishers used a fibreglass tank on their boat, while the most prevalent type of live-tank in the fall was the X-Actics™ box (see see Table XVI).

3.3.5.6 Water availability and lid cover with the live-tank

In the spring, 62.7% of the fishers waited until all traps had been retrieved before putting the lid completely on the live-tank, while 90.9% of the fall fishers had the lid on completely during fishing and also on the return trip to the wharf. Most fishers (83.1%) in the spring season waited until after fishing to fill the tank with water, while the majority (72.7%) of fall fishers had no water in the tank at any time (see Table XVII).

3.3.5.7 Overall handling of lobsters

After being removed from traps, 27.9% of the market-sized lobsters in the spring were generally tossed rather than placed into the temporary holding units. In the fall, the proportion of lobsters tossed into the temporary holding units was 18.2% (see Table XVIII). However, these two proportions were not significantly different.

Table XV. Holding systems used on fishing boats for temporary storage of lobsters before and after size-grading, during the 1997 spring, fall season, and overall in PEI.

HOLDING SYSTEM	SPRING		FALL		TOTAL	
	BEFORE GRADING	AFTER GRADING	BEFORE GRADING	AFTER GRADING	BEFORE GRADING	AFTER GRADING
TOTE	69.8%	96.2%	54.5%	81.8%	67.1%	93.8%
CRATE	0%	3.8%	45.5%	18.2%	7.8%	6.2%
PVC	1.9%	0%	0%	0%	1.6%	0%
X-ACTICS™	5.7%	0%	0%	0%	4.7%	0%
NONE	16.9%	0%	0%	0%	14.1%	0%
OTHER	5.7%	0%	0%	0%	4.7%	0%
TOTAL	100% (n=53)	100% (n=53)	100% (n=11)	100% (n=11)	100% (n=64)	100% (n=64)

Table XVI. Tank systems used during the 1997 spring and fall lobster fishing seasons in PEI.

LIVE-TANK SYSTEM	SPRING	FALL	OVERALL
FIBREGLASS	32 (71.1%)	4 (36.4%)	36 (64.3%)
X-ACTICS™	10 (22.2%)	5 (45.4%)	15 (26.8%)
NONE	3 (6.7%)	1 (9.1%)	4 (7.1%)
UNDER-DECK	0 (0%)	1 (9.1%)	1 (1.8%)
TOTAL	45	11	56

Table XVII. Lid cover and water availability on fishing boats during and after fishing, in the 1997 spring and fall seasons in PEI.

FISHING PRACTICE	SPRING		FALL		TOTAL	
	DURING FISHING	AFTER FISHING	DURING FISHING	AFTER FISHING	DURING FISHING	AFTER FISHING
LID						
completely on	37.3%	62.7%	90.9%	90.9%	45.7%	67.1%
3/4 on	10.2%	5.1%	0%	0%	8.6%	4.3%
half on	30.5%	23.7%	0%	0%	25.7%	20.0%
none	22.0%	8.5%	9.1%	9.1%	20.0%	8.6%
WATER						
flow-through	0%	3.4%	9.1%	9.1%	1.5%	4.3%
poured	11.9%	0%	9.1%	0%	11.4%	0%
stagnant	35.6%	83.1%	9.1%	9.1%	31.4%	71.4%
none	52.5%	13.5%	72.7%	81.8%	55.7%	24.3%

Table XVIII. Percentage of lobsters being placed versus being tossed from the traps to the temporary storage units on board fishing boats during the 1997 spring and fall seasons in PEI.

SEASON	PLACED	TOSSSED	TOTAL
SPRING	72.1%	27.9%	100%
FALL	81.8%	18.2%	100%
OVERALL	74.1%	25.9%	100%

3.3.5.8 Time on board fishing boats

The maximum amount of time lobsters spent on board fishing boats was significantly different between the spring and the fall seasons, with longer maximum time on board in the fall season ($P<0.001$). More than 62% of the time, the maximum time in the spring was between 4 h to 6 h, while this proportion was 27.3% in the fall. No lobster spent over 8 h on a boat in the spring, while 36% did in the fall (see Table XIX).

3.3.6 Transportation data

3.3.6.1 Transportation vehicles

A significant difference was present in the different vehicles used between spring and fall seasons to transport lobsters from wharves to processing plants ($P< 0.001$). During the spring, the vehicles mostly used consisted of trucks with refrigerated transportation compartments, while in the fall closed trucks without refrigeration were mostly used (see Table XX).

3.3.6.2 Transportation interval - travelling and shipping intervals

Although 37.5% of the travel intervals were less than 1 hour in the spring, 7.5% were in the range of 4 to 6 hours (see Table XXI). During sampling of the fall season, all transportation intervals were less than one hour. In the spring, 12.5% of the lobsters spent over 6 hours in transportation vehicles (shipping interval). The shipping interval in the fall was most commonly between 1 h to 2 h,

Table XIX. Maximum and minimum periods lobsters spent on board fishing boats during the 1997 spring and fall seasons in PEI.

TIME	FISHING SEASON		
	SPRING	FALL	OVERALL
MAXIMUM¹			
<2 h	0 (0%)	0 (0%)	0 (0%)
2-4 h	0 (0%)	0 (0%)	0 (0%)
4-6 h	28 (62.2%)	3 (27.2%)	31 (55.4%)
6-8 h	17 (37.8%)	4 (36.4%)	21 (37.5%)
8-10 h	0 (0%)	4 (36.4%)	4 (7.1%)
>10 h	0 (0%)	0 (0%)	0 (0%)
MINIMUM²			
<2 h	44 (97.8%)	10 (90.9%)	54 (96.4%)
2-4 h	1 (2.2%)	1 (9.1%)	2 (3.6%)
4-6 h	0 (0%)	0 (0%)	0 (0%)
6-8 h	0 (0%)	0 (0%)	0 (0%)
8-10 h	0 (0%)	0 (0%)	0 (0%)
>10 h	0 (0%)	0 (0%)	0 (0%)

¹ The maximum time represented the time period from when the first lobster was caught until the boat arrived at the wharf.

² The minimum time represented the time period from when the last lobster was caught until the boat arrived at the wharf.

Table XX. Types of transportation vehicles used between wharves and processing plants, during 1997 spring and fall sampling in PEI.

VEHICLE	DISTRIBUTION (%)		
	SPRING	FALL	OVERALL
DIRECT ¹	7 (17.5%)	0 (0.0%)	7 (14.3%)
REFRIGERATED TRUCK	26 (65.0%)	0 (0.0%)	26 (53.1%)
PICK-UP TRUCK	3 (7.5%)	3 (33.3%)	6 (12.2%)
CLOSED TRUCK ²	1 (2.5%)	6 (66.7%)	7 (14.3%)
UNKNOWN	3 (7.5%)	0 (0.0%)	3 (6.1%)
TOTAL	40 (100%)	9 (100%)	49 (100%)

¹ Direct meant that no vehicle was used; the lobsters were landed directly at the processing plants.

² A closed truck consisted of a truck without refrigeration unit in the transportation compartment.

Table XXI. Travelling and shipping intervals (in hours), during the 1997 spring and fall sampling in PEI.

HOURS	INTERVAL DISTRIBUTION (%)					
	SPRING		FALL		OVERALL	
	TRAVEL ¹	SHIPPING ²	TRAVEL ¹	SHIPPING ²	TRAVEL ¹	SHIPPING ²
DIRECT ³	7 (17.5%)	7 (17.5%)	0 (0.0%)	0 (0.0%)	7 (14.3%)	7 (14.3%)
<1	15 (37.5%)	6 (15.0%)	9 (100%)	6 (66.7%)	24 (49.0%)	12 (24.5%)
1 - 2	10 (25.0%)	3 (7.5%)	0 (0.0%)	3 (33.3%)	10 (20.4%)	6 (12.3%)
2 - 4	5 (12.5%)	10 (25.0%)	0 (0.0%)	0 (0.0%)	5 (10.2%)	10 (20.4%)
4 - 6	3 (7.5%)	8 (20.0%)	0 (0.0%)	0 (0.0%)	3 (6.1%)	8 (16.3%)
6 - 8	0 (0.0%)	3 (7.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (6.1%)
>8	0 (0.0%)	2 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (4.1%)
nk	0 (0.0%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.0%)
TOTAL	40 (100%)	40 (100%)	9 (100%)	9 (100%)	49 (100%)	49 (100%)

¹ Travel interval refers to the time interval between the wharves and the processing plants.

² Shipping interval corresponds to the total amount of time the shipment spent in the transportation vehicle.

³ Direct meant that no interval was calculated; lobsters were landed directly at the processing plants.

nk = unknown interval

with a proportion of 66.7%. Overall, 14.3% of the groups of lobsters were landed from the fishing boats directly at the processing plants, resulting in no shipping interval.

3.3.6.3 Ice with transportation

Whenever ice was used around lobster crates or totes, it was freshwater ice. Table XXII shows that 60% of the groups of lobster in the spring were shipped to the processing plants without ice, and in the fall season 66.7% of the groups of lobsters being transported between wharfs and processing plants were without ice.

3.3.6.4 Air temperature during road transport

The average temperature was 16.2 °C (± 3.1 °C) in the spring, and 21.5 °C (± 2.8 °C) in the fall. A significant difference was recorded between the mean air temperature of the two seasons.

3.3.6.5 Wind velocity during road transport

It was significantly windier in the fall fishing season than in the spring ($P=0.038$). In the spring, 86.3% of the time, the wind was categorized as being 'light,' while this proportion was 50% in the fall. The other 50% in the fall was the category 'moderate' which was observed only 10.3% of the time in the spring.

Table XXII. Use of freshwater ice in the vehicle shipping compartment during road transportation between wharves and processing plants in PEI during the 1997 spring and fall seasons, and overall.

	DISTRIBUTION (%)				
	WITH ICE	NO ICE	DIRECT ¹	UNKNOWN	TOTAL
SPRING	8 (20.0%)	24 (60.0%)	7 (17.5%)	1 (2.5%)	40 (100%)
FALL	3 (33.3%)	6 (66.7%)	0 (0.0%)	0 (0.0%)	9 (100%)
OVERALL	11 (22.5%)	30 (61.2%)	7 (14.3%)	1 (2.0%)	49 (100%)

¹ Direct meant that the lobsters were landed directly at the processing plants.

3.4 Discussion

Only the handling and fishing practices that showed significant and interesting results will be discussed. These included the lobster-level factors gender, size, physiological parameters and vigour, the environmental conditions, and the boat-level factors related to traps and bait, holding and live-tank systems, and finally the overall lobster handling. All remaining factors, including all transportation condition factors, did not show any substantial results and will not be discussed further.

The uneven distribution of wharves among the spring zones was due in part to random selection of wharves and also to the higher number of wharves located in Zone B. Because of the variation in sample sizes in the different zones, caution must be taken when comparing zones.

3.4.1 Gender

Ennis (1978) found that the male-female ratio of large lobsters when sampled by scuba divers was 1:1, but when estimated through trap capture, the ratio was 3:1 in favor of male lobsters. The overall sex ratio of all tagged lobsters included in this study was about 52% females vs. 48% males. Higher proportions of females were found in two zones (B and C) with 57% and 67.1%, while the other two zones showed lower proportions of females with 43.2% and 40.6%. Assuming a normal distribution of the sex ratio among all hatched lobster eggs, and equal survival rates among female and male lobsters to market size, a 50% male, 50% female population should be available for harvest.

Federal regulations require that all females carrying eggs on the ventral surface of their tail (ovigerous females) must be returned to the water, and the removal of their eggs is prohibited (Miller, 1995). By protecting ovigerous females, fewer females should be landed, since on average female lobsters will carry eggs externally for approximately a year (Waddy *et al.*, 1995), thus increasing the likelihood of catching more males at any point in time. The reason for the higher proportion of females landed in Zones B and C is still unclear, but could reflect a competitive behaviour among males, as suggested by Campbell (1992), or perhaps a difference in the feeding behaviour of male versus female lobsters.

In the early part of the fall season, a significant proportion of lobsters are in post-ecdysis. Female lobsters will mate shortly after the ecdysis (Talbot and Helluy, 1995). A higher proportion of males caught in this period could reflect shelter-restricted behaviour of females who recently molted and then mated. Waddy and Aiken (1990) reported a higher relative activity of mature lobster males than females. This behavioural difference could also explain a gender ratio of landed lobsters favoring males over females. Zone D, which represented the fall sampling, was the zone with the highest male to female ratio with almost 1.5 males per female. The significance of the gender difference in landed lobsters is uncertain.

3.4.2 Size

Some of the findings of this study regarding lobster size contradict the accepted theory that because males grow to larger size, they tend to dominate

the sex ratio in larger size populations (Cobb, 1995). According to Cobb (1995), both sex and size are two factors that could generate different lobster behaviours when facing fishing traps and this study corroborated those conclusions.

Pringle and Burke (1993) suggested that size differences in neighboring populations of lobsters could reflect a more recent exploitation of deeper water, overwintering sites and different habitats. A newer fishery should yield lobsters of bigger average size, whereas a fishery that has been established for a longer period usually consists of lobsters of smaller average size. As the fishery gets 'older,' fewer of the older and bigger animals remain in the wild, and most of the catch will contain new recruits, or lobsters in their first year of meeting the minimum legal fishing size. However, there are no reasons to believed this would be applicable for Prince Edward Island.

3.4.3 Physiological parameters

Some haemolymph parameters have been used to define lobster health (Jussila *et al.*, 1997; MacKenzie *et al.*, 1997b). In this study, 95% of the lobster population had THP levels measured on the boats were between 18.6 g/L and 99.8 g/L. The levels of THP reported by MacKenzie *et al.* (1997a) were within the range of these data, with a mean value of 67.8 g/L, while the values reported by Chen and Chia (1997) in the mud crab (*Scylla serrata*) were also similar, at 81.0-88.4 g/L.

MacKenzie *et al.* (1997b) reported a mean value for THC in their laboratory study of 12.6×10^6 haemocytes/ml while the value reported by Cornick

and Stewart (1978) was slightly higher at 18.1×10^6 haemocytes/ml. These two values are also within the mean $\text{THC} \pm 2 \text{ SD}$ (95% of the population) reported in this study (5×10^6 haemocytes/ml to 50.5×10^6 haemocytes/ml). Jussila *et al.* (1997) reported a range for THC in western rock lobster (*Panulirus cygnus*) of 2.5 to 15.9×10^6 haemocytes/ml, with the highest mean THC in lobsters freshly arrived at the factory tanks, and suggested stress from handling or exposure to air as causative factors for the high mean THC.

Lobster THP levels are influenced by the time of year, the molting cycle, the water temperature and probably by many other factors including diet, size and gender (Ennis, 1973; Chen and Chia, 1997; Paterson and Spanoghe, 1997; MacKenzie *et al.*, 1997a). Immediately following the completion of the ecdysis, lobsters will ingest and absorb substantial volumes of seawater to expand their volume to often 50% greater than they were prior to the ecdysis (Aiken and Waddy, 1992), which dilutes the haemolymph, resulting in lower THP levels and lower THC. Because THP levels are affected by the molting cycle, and since the molt is regulated in part by water temperatures, variations in protein levels were expected during this study as water temperature increased. This study demonstrated significantly increasing THP levels during the spring season, followed by a significant decrease in the fall. The fall fishing season of Prince Edward Island is timed to occur after most newly molted lobsters have achieved stage C of the molting cycle. Chen and Chia (1997) reported the lowest protein levels for the mud crab (*Scylla serrata*) during stage B, and the highest levels during stages D₂ and D₃. High level of feeding activities could be necessary to

enable lobsters to recover from the immediate low post-molt condition, as suggested by varying serum protein in field-captured lobsters (Ennis, 1973).

Because many external factors can affect the THP and THC levels post-landing, the values obtained when lobsters were taken onto the boats were probably the most representative assessments of the natural situation.

Female lobsters had significantly lower THP levels than males at every handling point of the industry during the spring season: boat, wharf, point of entry to the processing plant, and exiting the plant. MacKenzie *et al.* (1997a) only reported a weak difference ($P<0.1$) between male and female American lobster haemolymph protein levels held under laboratory conditions. Chen and Chia (1997) reported no significant difference in THP levels between male and female mud crabs (*Scylla serrata*). During the fall, male lobsters had significantly higher THP levels than females only when assessed at entry to processing plants.

Another important finding was the evidence of dehydration in lobsters kept out of the water, demonstrated by increasing THC from boats to wharves, and between additionally, from boats to arrival at processing plants. Perhaps lobsters kept out of the water for extended periods are losing considerable amounts of body fluids. This loss of fluids could translate into haemo-concentration, resulting in higher THC. The increase in THP levels from boats to wharves and to processing plants was not as obvious as the trend for the THC. Dehydration has been demonstrated in prawns (*Penaeus japonicus*) to be up to 0.75% loss of body weight per hour of exposure to air, at 75-85% relative humidity (Samet *et al.*, 1996). Newsome *et al.* (1994) suggested that spraying

red swamp crawfish (*Procambarus clarkii*) with seawater could probably protect them against dehydration through evaporation, and may also help replace some of the body fluids lost.

During the spring season, a significant difference in the THC values between gender was seen only at the wharves, with males having higher counts. For the fall season, significant differences in THC between male and female lobsters were observed on the boats and at the time of entry into the processing plants; males having higher counts than females at the plants, but lower at the boats. Overall, no consistent patterns in the THC according to sex were recorded, similar to the findings of Jussila *et al.* (1997) on rock lobsters (*Panulirus cygnus*). Cornick and Stewart (1978) and MacKenzie *et al.* (1997b) did not assess sex differences in THC, although Cornick and Stewart looked at differential haemocyte counts and reported no significant difference according to gender. Because female lobsters do not molt as often as males, some variation between gender in both THP and THC values could be present prior to, during, and shortly after ecdysis, which could explain some of the results of this study.

The wide range of THP and THC values observed may be helpful, if certain factors affecting these ranges can be identified, and therefore used as indicators of health. Further studies of factors influencing the range instead of the mean values of either THP or THC, are required. However, the identification of such factors must be addressed.

3.4.4 Lobster vigour

The initial assumption was that lobster health status would start decreasing only after lobsters entered the traps. Zone D (fall lobster fishing season) had a significantly higher proportion of weak lobsters on the boats than Zones B and C. However, this proportion of weak lobsters did not significantly increase from the time of the assessments on fishing boats to the assessments at the time of entry into processing plants. Most vigour loss occurred between wharves and processing plants, and not between boats and wharves. Perhaps these vigour losses were induced by injuries inflicted on the boats and their effect was not detected until later.

3.4.5 Weather

Lobsters are sensitive to fresh water exposure (Jury *et al.*, 1994; Ennis, 1995; McMahon, 1995), and therefore, heavy exposure to rain will likely be detrimental for lobsters, especially for prolonged exposure times. Furthermore, exposure to sunshine could result in more rapid drying of the external surface of the lobsters, resulting in a loss of body fluids. Newsome *et al.* (1994) suggested that water is lost more rapidly than other components of the haemolymph, and that the haemolymph concentration should increase.

3.4.6 Fishing practices

Stress from harvesting and handling of western rock lobsters has been blamed for productivity losses in the lobster (*Panulirus cygnus*) industry of

Western Australia (Paterson and Spanoghe, 1997). Similarities with the Canadian lobster fishery are most likely, and the various fishing practices should also have profound impacts on further industry losses.

3.4.6.1 Setting of traps and bait

Colder water temperatures at greater depths might have different impact on lobster health than warmer waters from shallower bays. Greater hydrostatic pressure difference is experienced by lobsters from deeper traps when brought to the surface. Perhaps this change in pressure removes some of the oxygen from the haemocyanin, resulting in anoxia.

The type of bait and its composition and quality, may physiologically affect lobsters. Crawfish trap catch was highly influenced by many factors, including bait type, quality and quantity (Romaire, 1995). However, Addison and Bell (1997) reported that reduced bait attractiveness had less influence on catch rates than behavioural interactions. Different trap attraction rates were evaluated when mussels, sea urchins, cattle hocks, animal guts and diesel oil were used as bait in spiny lobster (*Panulirus* sp.) traps (Mohan Rajan *et al.*, 1995), with mussels and diesel oil, being respectively the most effective baits recommended by the authors. Mussels as bait could represent an interesting alternative bait in Prince Edward Island, with the important number of mussel farms and processing plants; mussels being culled at harvest could be used by lobster fishers. Unfortunately, correlations between types of bait used and numbers of lobsters caught were not investigated in this study. A formulated bait

(commercial feed) could also represent another alternative for the fall lobster fishery; crawfish fishers typically use 'fish bait' in cold waters (<20 °C), but when fishing in warmer waters (>20 °C), they either use 'formulated bait' or a combination of fish and formulated baits (Romaire, 1995).

3.4.6.2 Holding system and live-tank system

Lobsters, unlike finfish, have respiratory capability out of the water. To extract oxygen from the air, their gills must be kept wet. The tolerance of aquatic crustaceans to exposure to air is greatly increased by cooling the animals, and by having a high relative humidity (Samet and Nakamura, 1997). Therefore, having ice in the live tank can be an advantageous alternative, when no water is used. However, when water stays stagnant for long periods, oxygen is depleted, and lobsters become hypoxic. Lobsters usually survive for longer periods if they are kept out of the water, in a cool environment where their gills stay wet, compared to maintenance in stagnant water with no aeration. Keeping aquatic crustaceans dry is harmful, especially if they are exposed to wind or direct sunshine; the gills eventually collapse if the relative humidity drops beyond a critical value for an extended period (Samet and Nakamura, 1997). The use of a lid offers some protection from direct sunlight or rain, and can help maintain the relative humidity high and constant in the live tank. The lid may also help in maintaining the live tank inside temperature constant. Sudden and drastic changes in water temperature are stressful on lobster, and have strong influence on their behaviour (Crossin *et al.*, 1998), and most likely on their health status.

Unfortunately, the water quality of the live-tanks was not assessed during this study; its effect could not be documented.

3.4.6.3 Overall handling of lobsters

It is logical to expect a higher proportion of lobster injuries on boats where fishers were less careful with their product. No statistical difference was found between the two seasons in the overall handling of lobsters from the traps to the temporary holding unit, partly due to the small sample size in the fall (n=11). The power of detection was 6.6%, indicating that with this sample size, even if a difference between seasons existed, it would be detected only 6.6% of the time.

3.5 Conclusions

The objectives of this chapter were achieved with a detailed quantitative assessment of post-harvest losses in the Canadian lobster, and with a thorough examination and description of handling and fishing practices in Prince Edward Island during the spring and fall fishing seasons of 1997. Finally, a complete comparison of the two fishing seasons of Prince Edward Island was performed to describe where and when post-harvest losses occurred.

THP levels varied greatly according to gender and the time of year, and therefore they represent important considerations when using total protein as an indicator of lobster health. Pound operators and managers should be encouraged to build a database of reference values for both seasons separately, and should avoid combining data from one season to the other. THC showed

significant increases between boat assessment and wharf assessment. When lobsters are kept out of the water, they lose body fluids, resulting in haemo-concentration. Perhaps this haemo-concentration was reflected by the increase in the THC. This makes comparisons between groups of lobsters difficult to interpret. THP levels did not demonstrate dehydration as well as THC did. Serum protein levels should not be considered reliable indicators of health at the population level and their usefulness at the individual lobster level should be interpreted with caution, according to time of year. THC may be a more reliable health indicator than total protein, if the dehydration phenomenon could be controlled or considered in the interpretation. Perhaps using both THP and THC together may be more helpful in determining lobster health than using these indices individually. The influence of dehydration on lobster health and on other haemolymph parameters, and the possible correlations between these haemolymph parameters and stages of the molt cycle, gender, and the reproduction cycle require further investigations to make more accurate conclusions.

Attempting to follow lobsters from fishing boats through wharves and processing plants is a logistical challenge for evaluation of lobster health or productivity. In many field studies, these logistical problems have limited the ability to obtain valid results. This study enabled the investigators to identify the frequency with which a series of different fishing practices, and transportation conditions was used. By using epidemiological methods, a model predicting factors related to lobster survival could eventually be built.

4. ANALYTICAL ASSESSMENT OF HANDLING, FISHING PRACTICES, AND TRANSPORTATION CONDITIONS ON LOBSTER HEALTH

4.1 Introduction

Among Canadian fisheries, the lobster (*Homarus americanus*) fishery is one of the most important, both in volume and in landed value. It consists primarily of a specialized inshore small boats fishery (Pringle and Burke, 1993).

Paterson & Spanoghe (1997) suggested that sampling lobsters at various points of handling should yield information on stressors influencing lobster health. Variations in lobster health, before and after short- to long-term holding, are hypothesized to be associated with conditions on fishing vessels, and perhaps wharf-level factors.

Through questionnaires assessing handling and fishing practices on board fishing boats during the spring and fall fishing seasons of 1997 in Prince Edward Island and transportation conditions between fishing wharves and processing plants, several potential risk factors for productivity losses in the Canadian lobster industry were identified (see Appendices M and N). These factors covered some of the crew and boat specifications, i.e., crew size, years of experience, and boat size. They also included the environmental variables rain, sunlight, wind, wave, water and air temperatures. Among fishing practices, possible risk factors included: trap setting configuration and depth at which traps were set, overall lobster handling by the crew, type of bait, contact among lobsters before grading, type of temporary storage unit and holding tank,

separation and partition before grading, water and lid availability for holding tanks, packing lobsters over and lobster dumping (packing over was defined as completely emptying a crate or tote by taking each lobster one by one and re-packing them into another container, while dumping lobsters was defined as transferring the entire crate or tote into another container all at once), having lobsters loose on the deck, and maximum and minimum periods spent on boats. Transportation conditions included the following risk factors: type of vehicle, presence of ice during transportation, type of shipping unit, outside air temperature and other weather conditions, interval between wharves and processing plants, and maximum time shipments stayed in transport vehicles. For further details of the fishing practices and transport conditions, see Chapter 3, section 3.3.

The objective of this study was to develop an epidemiological model to assess the impact of different fishing and handling practices previously identified as potential determinants of the health and quality of lobsters upon arrival at processing plants.

4.2 Materials and methods

4.2.1 Data collection and variable selection

A list of fishing practices, handling practices, and transportation conditions which may significantly impact lobster health before holding, after holding, or prior to processing and marketing was obtained from questionnaires completed by investigators during a study conducted from May 1997 to August 1997 in

Prince Edward Island, Canada (Data collection has been described in detail in Chapter 3, sections 3.2.5 and 3.2.6, and Appendices M and N). Fishing and handling practices were monitored on 64 boats, while transportation conditions were assessed on 49 different vehicles (the difference is due to the fact that on 30 occasions, both boats assessed sold to the same buyer, and therefore were transported in the same vehicle). From 64 groups of lobsters assessed during this study, 17 groups were rejected due to missing information at the processing plants. The outcome, lobster vigour upon arrival at the processing plant, was assessed on a total of 2,191 lobsters from 47 fishing boats. Variables assessed with the fishing boat and transportation questionnaires were either continuous, categorical or dichotomous variables. Continuous variables are variables taking values which are intrinsically numerical and measurable, categorical variables are qualitative variables which have values or categories that are nominal or ordinal, while dichotomous variables are categorical variables which can only take on two values (Fisher and van Belle, 1993). The use of continuous variables in a statistical model implies a linear relationship between the independent and dependent variables (Kleinbaum *et al.*, 1988). If this was not reasonable (if the independent variable showed a tendency to group around discrete values), then the variable was converted to a categorical variable with two (or more) levels.

The outcome variable for the model was a dichotomous variable describing the vigour of each lobster, assessed at arrival at the processing plant. Each lobster was given a score of '1' for any loss of vigour, or a score of '0' if the animal was considered to have a normal level of liveliness.

4.2.2 Statistical analysis

The dataset was transferred into a statistical software package (STATA™ version 5.0, Stata Corporation, College Station, Texas, USA, 1996). Dummy variables were created for every categorical variable. After transformation of every categorical variable into dichotomous variables and exclusion of variables pertaining to canner-sized lobsters, 49 variables were retained (including the outcome variable). Due to the limited number of boats sampled in the fall, only data from boats from the spring season were used in developing the models. The total number of groups of lobsters included in the analyses was then reduced to 38. Unconditional associations between the outcome (lobster vigour score at arrival at the processing plant) and the predictors were estimated by Chi-square tests for dichotomous variables and by T-tests for the continuous variables. For all analyses, observed associations were considered significant when $P \leq 0.05$.

Variables with significant unconditional associations with the dependent variable (lobster vigour) were selected for inclusion in a multiple variable model building process. The model used was a logistic regression model estimated by using a generalized estimating equation (GEE) procedure (Liang and Zieger, 1986). The model assumed a binomial error distribution, and used a logit link function with an exchangeable correlation structure for the correlations among lobsters within a boat.

A forward stepwise procedure under the control of the investigator (i.e., not computer generated) was used to identify variables having important

associations with lobster vigour at the processing plants. Initially, single variables with significant association were identified. Then, all possible two-way interactions among these individually significant variables were explored. Finally, three way interactions were considered for inclusion in the model. Models were compared using the deviance statistic, and the model with the lowest deviance was selected. The fit of the model was assessed using a Hosmer-Lemeshow goodness-of-fit test. This model building procedure was used to build two separate models that included only boat-level factors.

4.3 Results

4.3.1 Measures of association

Three continuous variables (date, depth_1 and depth_2) and 19 of the 39 dichotomous variables were found to be significantly associated with lobster vigour (see Tables I and II), and kept for further analysis. The vigour of lobsters assessed directly on boats was significantly associated with vigour upon arrival at the processing plants. However, because only three cases of decreased vigour on boats were observed and one of them was then re-observed at the plant (i.e., 33.3% of the lobsters with decreased vigour on the boats had also a decreased vigour at the plant), this variable was not kept as predictor for further analysis.

Table I. Description of the continuous variables with their corresponding means for normal versus decreased lobster vigour. The *P*-values were obtained by T-tests.

VARIABLE	DESCRIPTION	<i>P</i> - VALUE	MEAN	
			NORMAL VIGOUR ¹	DECREASED VIGOUR ¹
DATE	date of the boat sampling (# of days from January 1)	0.026	162.9	166.3
WATER_T	surface water temperature (°C)	0.181	7.9	8.6
NO_TRAPS	number of traps hauled	0.111	289	300
DEPTH_1	maximum depth at which the traps were set (m)	0.001	17.6	14.9
DEPTH_2	minimum depth at which the traps were set (m)	0.003	5.4	3.5
WEIGHT	individual weight as measured at the wharf (kg)	0.381	0.58	0.63
LENGTH	individual length as measured on the boat (mm)	0.414	88.2	89.4
PROTEIN	total haemolymph protein, on the boat (g/L)	0.190	77.4	84.8
HEMOCYTE	total haemocyte counts, on the boat (x 10 ⁶ haemocytes/ml)	0.422	198.3	221.3

¹ Normal and decreased vigour as assessed at the processing plant with the dichotomous variable VIGOUR_3.

Table II. Description of the dichotomous variables with their frequency distributions for normal versus decreased lobster. The *P*-values were obtained by Chi-square tests. RR is the relative risk associated with each variable.

VARIABLE	DESCRIPTION		PROPORTION WITH DECREASED VIGOUR AT THE PLANT (%)	P-VALUE	RR
VIGOUR_3 ¹	lobster liveliness, as assessed at the plant	normal decreased	1,214 (97.20%) 35 (2.8%)	na	na
CREW_34	size of the crew on the boat, including the captain	2 or + 3 or +	28/776 (3.61%) 7/473 (1.48%)	0.027	2.4
EXP_LOT	years of fishing experience of the captain	21 yrs or + 20 yrs or -	27/652 (4.14%) 8/597 (1.34%)	0.003	3.0
MXAIR_10	maximum daily air temperature	11 °C or + 10 °C or -	20/340 (5.88%) 15/909 (1.65%)	<0.001	3.6
MINAIR_9	minimum daily air temperature	10 °C or + 9 °C or -	21/412 (5.10%) 14/837 (1.67%)	0.001	3.1
MOD_WIND	wind strength while fishing	moderate-strong calm-light	2/201 (1.00%) 33/1,048 (3.15%)	0.090	0.3
RAIN_YN	raining during fishing	yes no	21/239 (8.79%) 14/1,010 (1.39%)	0.000	6.3
SUN_YN	sunshine during fishing	yes no	14/972 (1.44%) 21/277 (7.58%)	<0.001	0.2
BEFWATER	water available in the live tank, while fishing	no yes	26/795 (3.27%) 9/454 (1.98%)	0.185	1.7
AFTWATER	water available in the live tank, after fishing	yes no	29/1,004 (2.89%) 6/245 (2.45%)	0.709	1.2
BAIT_1	fresh gaspereaux used as bait	yes no	4/400 (1.00%) 31/849 (3.65%)	0.008	0.3
BAIT_3	fresh mackerel used as bait	yes no	29/700 (4.14%) 6/549 (1.09%)	0.001	3.8
BAIT_5	fresh herring used as bait	yes no	2/127 (1.57%) 33/1,122 (2.94%)	0.377	0.5
BAIT_7	fresh flatfish used as bait	yes no	6/47 (12.77%) 29/1,202 (2.41%)	<0.001	5.3
TANK_1	fibreglass box as live tank	yes no	5/249 (2.01%) 30/1,000 (3.00%)	0.396	0.7
TANK_2	x-actic box as live tank	yes no	30/907 (3.31%) 5/342 (1.46%)	0.078	2.3
HOLD_2	plastic tote used for holding unit before grading	yes no	31/817 (3.79%) 3/317 (0.95%)	0.012	4.0
HOLD_6	wooden box used for holding unit before grading	yes no	0/95 (0.00%) 34/1,039 (3.27%)	0.073	na
A_HOLD_2	plastic tote used for holding unit after grading	yes no	35/1,176 (2.98%) 0/73 (0.00%)	0.135	na

¹ OUTCOME

Table II. (cont.)

VARIABLE	DESCRIPTION	FREQUENCY DISTRIBUTION IF VIGOUR IS DECREASED AT THE PLANT (%)		P-VALUE	R.R.
PART_2	partitioned holding unit before grading	yes no	0/115 (0.00%) 34/1,019 (3.34%)	0.047	na
CONTAC_4	physical contact among lobsters before being banded	yes no	34/895 (3.80%) 0/239 (0.00%)	0.002	na
HAND_2	overall lobster handling procedure on the boat	tossed placed	31/859 (3.61%) 4/322 (1.24%)	0.033	2.9
MOD_WAVE	moderate to high waves vs. small or calm sea	moderate-strong calm-small	14/212 (6.60%) 21/1,037 (2.03%)	<0.001	3.3
DTRP3	combination of multiple, single or double trap setting	yes no	35/1,176 (2.98%) 0/73 (0.00%)	0.135	na
LID_BEF	presence of a lid on the live tank while fishing	yes no	34/1,083 (3.14%) 1/166 (0.60%)	0.065	5.2
TIME_MAX	maximum time one lobster spent on the boat	4-6 hours 6-8 hours	23/730 (3.15%) 12/519 (2.31%)	0.377	1.4
TIME_MIN	minimum time one lobster spent on the boat	> 2 hours 2-4 hours	35/1,193 (2.93%) 0/56 (0.00%)	0.194	na
PACK_2	packing over of the lobsters at the wharf	yes no	3/283 (1.06%) 32/966 (3.31%)	0.043	0.3
TRUCK_2	transportation between wharf & plant in closed compartment	yes no	4/108 (3.70%) 10/986 (1.01%)	0.018	3.7
DIRECT	lobsters landed directly at the plant, no transport vehicle	yes no	1/239 (0.42%) 13/855 (1.52%)	0.180	0.3
MOD_TWIN	wind strength during road transportation	moderate-strong calm-light	21/286 (7.34%) 14/963 (1.45%)	<0.001	5.1
T_TEMP18	air temperature during road transportation	19 °C or + 18 °C or -	29/506 (5.73%) 6/743 (0.81%)	<0.001	7.1
SEX	gender of the lobsters	female male	23/672 (3.42%) 12/570 (2.11%)	0.162	1.6
GAFF	gaffkernia test result, on the boat (for <i>Aerococcus viridans</i>)	positive negative	2/23 (8.70%) 12/494 (2.43%)	0.070	3.6
VIGOUR	lobsters liveliness, as assessed on the boat	decreased normal	1/3 (33.33%) 34/1,245 (2.73%)	0.001	12.2
WOUND	wound or active lesion, as assessed on the boat	present absent	5/145 (3.45%) 30/1,103 (2.72%)	0.617	1.3
NOR_CLAW	quality of the claws, as assessed on the boat	normal abnormal	29/1,051 (2.76%) 6/198 (3.03%)	0.832	0.9
NOR_LEG	quality of the legs, as assessed on the boat	normal abnormal	33/1,162 (2.84%) 2/87 (2.30%)	0.768	1.2
NOR_ANT	quality of the antennae, as assessed on the boat	normal abnormal	29/1,061 (2.73%) 6/188 (3.19%)	0.726	0.8
NOR_BODY	quality of the overall body, as assessed on the boat	normal abnormal	33/1,152 (2.86%) 2/97 (2.06%)	0.645	1.4

4.3.2 Handling and fishing practices

4.3.2.1 Depth

The minimal and maximal depths at which traps were set showed significant unconditional associations with vigour status of the lobsters assessed upon arrival at processing plants. When vigour was below normal at the plant, the average minimal depth was 3.5 m, while the average was 5.4 m for lobster with normal vigour. A maximal depth average of 14.8 m for decreased vigour lobsters compared to 17.6 m for normal lobsters was observed. Both comparisons suggested that lobsters caught in shallower water were more likely to have reduced vigour (see Table I).

4.3.2.2 Crew

Boats with smaller crews or older captains tended to have a higher risk of producing low vigour lobsters. When landed from boats with crews of 2 members or less, lobsters had a chance of having loss of vigour upon arrival at the processing plants 2.4 times greater than when they were landed by larger crews. The chance of observing lobster vigour loss when landed by a captain with more than 20 years of experience was 3 times greater than if the captain had 20 years of experience or less (see Table II).

4.3.2.3 Weather and environment

A number of weather related variables had significant unconditional associations with lobster vigour. Warm weather, rain, sunlight, and rough

weather (waves) all increased the risk of low vigour. After dichotomization, both the maximum ($>10^{\circ}\text{C}$ or $\leq 10^{\circ}\text{C}$) and minimum ($>9^{\circ}\text{C}$ or $\leq 9^{\circ}\text{C}$) air temperatures had significant unconditional associations with lobster vigour at processing plants. When the maximum temperature was above 10°C , lobsters were 3.6 times more likely to experience loss of vigour at the plants than when the maximum daily temperature was 10°C or below. A similar situation was noted with the minimal daily air temperature, with relative risk of observing decreased lobster vigour at the plant of 3.1 when the minimal air temperature was above 9°C . When caught on rainy days, lobsters were 6.3 times more likely to have decreased vigour upon arrival at the plant than when they were on non-rainy days. Also significant was the exposure to sunlight, with relative risk for lobster decrease in vigour of 5.3 if landed on sunny days compared to cloudy days. When the waves were classified as moderate to strong, lobsters were 3.3 times more likely to suffer from loss of vigour at the plants compared to when waves were calm to minimal (see Table II).

4.3.3.4 Bait

Boats fishing with mackerel or flatfish baits were at higher risk of delivering lobsters with lower vigour, while boats fishing with gaspereaux bait landed livelier lobsters. When fished with mackerel, lobsters became almost 4 times more likely to experience loss of vigour, than when alternate baits were used. Compared to all other baits, gaspereaux had a protective effect: lobsters fished with gaspereaux were only 1/3 as likely to have reduced vigour. Finally,

lobsters fished with flatfish as bait were 5.3 times more likely to suffer loss of vigour upon arrival at the processing plant (see Table II).

4.3.2.5 Holding and handling

Rough handling, physical contact among lobsters prior to measuring, the absence of partitioned holding units, the use of plastic totes as temporary storage units, and packing lobsters over at the wharves, were all practices which induced higher risk for lobster vigour loss. Lobsters coming from boats where physical contact prior to measuring was possible had an 18 times greater chance of suffering from loss of vigour at the processing plants than if they were landed from boats where no physical contact occurred. To prevent physical contact among lobsters, some fishers used partitioned holding units, and when doing so, lobsters were approximately 8 times less likely to suffer decreased vigour at the processing plants.

Lobsters landed from boats where they were generally tossed from the traps to the temporary holding units were almost 3 times more likely to suffer from vigour loss at plants compared to lobsters that were placed into temporary holding units. If these temporary holding units were the traditional plastic totes, then lobsters were 4 times more likely to experience loss of vigour at processing plants than if other types of temporary storage units were used. Furthermore, the practice of packing over the lobsters once at the wharf showed that lobsters which went through this process were more than 3 times more likely to have decreased vigour at the processing plants than lobsters which did not go through

this process. All other handling and fishing practices did not show any significant unconditional association with the lobster vigour status at processing plants (see Table II).

4.3.3 Transportation conditions

Only three transportation variables individually showed statistically significant association with lobster vigour loss when assessed at arrival at processing plants: the use of closed compartment vehicles, warmer outside air temperatures, and windy conditions during transport between wharves and processing plants.

When the transport vehicle was a closed compartment truck, lobsters were almost 4 times more likely to have decreased vigour than if other types of vehicles were used. In the presence of moderate to strong winds during transportation, the proportion of lobsters which suffered from loss of vigour upon arrival at the processing plants was 7.3% compared to 1.5% if the winds were calm to light; lobsters transported during windier days were more than 5 times more likely to have vigour loss at the plants. Finally, if the outside air temperature was above 18 °C, lobsters became 7 times more likely to suffer vigour loss at the processing plants than if the air temperature was 18 °C or less. No other transportation conditions showed significant association with lobster vigour at the processing plants (see Table II).

4.3.5 Regression models

The Generalized Estimating Equation logistic regression model (GEE) identified four significant variables predicting lobster vigour at the processing plant: maximum depth at which traps were set, rain during fishing, handling of the lobsters from traps to temporary storage units, and the use of mackerel as bait (see Table III). Therefore, when controlling for other factors, lobsters landed from boats where mackerel bait was used instead of alternate baits were more than 7 times more likely to have decreased vigour at the processing plants. If landed on rainy days, lobsters were 6.3 times more likely to have decreased vigour than when landed on non-rainy days, when controlling for other factors. When the lobsters were generally tossed into the temporary holding units, they were 3.6 times more likely to experience vigour loss at the plant, when other factors were considered. Finally, lobsters caught in deeper waters were less likely to have loss of vigour at the processing plants than lobsters caught from shallower waters, with the chance of experiencing loss of vigour decreasing by 1.2 for every metre the depth was increased. This odds ratio of 1.2 for depth was also calculated when controlling for other variables. The deviance for this model was 240.81, the Pearson dispersion coefficient for the model was 1.03, with a total of 1,148 observations, and the Chi-square value was 36.56 ($P<0.001$). The standard errors were adjusted for clustering on the boat, due to the GEE model. The Hosmer-Lemeshow goodness-of-fit test yielded a Chi-square value of 8.11 with a P -value of 0.423 (critical value=15.507, $P\leq 0.05$, $df=8$), and therefore, it was concluded that the model fit the data reasonably well.

Table III. Results of the first generalized estimating equation (GEE) regression model with 95% confidence interval to predict lobster vigour at the processing plant.

VIGOUR	ODDS RATIO	Z	P> z	95% CONF. INTERVAL
MAX DEPTH	0.85	-3.008	0.003	-0.085 -0.018
RAIN	3.63	2.538	0.011	0.294 2.286
HANDLING	0.28	-1.977	0.048	-2.559 -0.011
MACKEREL	7.07	3.018	0.003	0.686 3.226

max depth: maximum depth at which the traps were set (m)
 rain: if it was raining during fishing
 water in tank: if there was water in the live tank during fishing
 handling: if the lobsters were placed (vs. tossed) from the traps to the temporary storage unit
 mackerel: if fresh mackerel was used as bait

A second model identified four significant variables, and one marginally significant variable: maximum air temperature $> 10^{\circ}\text{C}$, flatfish as bait, handling of the lobsters from traps to temporary storage unit, minimum depth at which traps were set, and the marginal variable was rain during fishing (see Table IV). When the effect of other variables was taken into account, the odds ratio for having decreased vigour was almost 9 when flatfish was used as bait compared to alternate types of bait, and the odds ratio for fishing on warmer days also approached 9. Lobsters tossed from traps to temporary holding units were more than 3 times more likely to suffer vigour loss than lobsters generally placed into the temporary holding units. For every metre of additional depth at which traps were set, lobsters became 1.3 times less likely to have decreased vigour at the processing plants when the effect of the other variables was considered. The deviance for this model was 257.54, the Pearson dispersion coefficient was 1.08, with a total of 1,181 observations, and a Chi-square value of 53.75 ($P<0.001$). The standard errors were also adjusted for clustering on the boat due to the use of the GEE logistic regression model. The Hosmer-Lemeshow goodness-of-fit test yielded a Chi-square value of 4.337 with a corresponding P -value of 0.826 (critical value=15.507, $P\leq 0.05$, $df=8$), and therefore again, the model seemed to fit the data adequately.

Table IV. Results of the second generalized estimating equation (GEE) regression model with 95% confidence interval to predict lobster vigour at the processing plant.

VIGOUR_3	ODDS RATIO	Z	P> z	95% CONF. INTERVAL
MAX AIR	4.69	3.211	0.001	1.825 12.024
FLATFISH	8.83	3.246	0.001	2.369 32.875
HANDLING	0.30	-2.348	0.019	0.108 0.818
MIN DEPTH	0.76	-2.583	0.010	0.159 0.980
RAIN	2.40	1.824	0.068	0.937 6.137

max air: if the maximum daily air temperature during fishing was above 10 °C
 flatfish: if fresh flatfish was used as bait
 handling: if the lobsters were placed (vs. tossed) from the traps to the temporary storage unit
 min depth: minimum depth at which the traps were set (m)
 rain: if it was raining during fishing

4.4 Discussion

More than 45% of all determinants showed significant crude association with the outcome. Having multiple observations for every cluster-level, i.e., fishing boat, can artificially increase the significance of many determinants (Kleinbaum *et al.*, 1988), and a conservative approach must be taken when drawing conclusions, especially with crude associations. Although the distribution of the event of concern was binomial (taking on only one of two values: normal or decreased vigour), the frequency of the event meant that the number of boats with decreased lobster vigour was sufficiently rare to have any statistical power, if the data had been collapsed to the boat level. Since, the data were clustered at the boat-level, an ordinary logistic regression would not account for this clustering (Kleinbaum *et al.*, 1988), and would likely overestimate the significance of predictor variables. The investigators wanted to keep individual lobsters as the unit of evaluation, so both lobster-level and boat-level factors could be considered. Consequently, a GEE logistic modelling approach was chosen. Adding the fall season only increased the variation of the data, and therefore the instability of the models; data from the fall were dropped.

When dealing with large biological datasets, it is sometime possible to obtain different models which can all be significant (Kleinbaum *et al.*, 1988). In this study, both models were significant, but the second model explained less of the variation of the data than the first one, with a greater deviance (257.8 versus 240.8 for the first model). Also, flatfish bait, one of the predictors included in the second GEE model was used for about 10% of the lobsters, which represented

lobsters from only one boat out of 38 (2.6%). Therefore, this second model, although significant, should not be considered as reliable as the first regression model and is therefore rejected.

The final GEE model included four significant predictors. The significant predictors for loss of vigour at arrival at the processing plant did not include any lobster-level factors, but only consisted of boat-level factors. Lobsters landed from boats using mackerel bait were more than 7 times more likely to suffer from loss of vigour when arriving at the processing plant compared to lobsters landed from boats using alternative baits, when controlling for the other variables. The biological or physiological explanation for this phenomenon is not clear. Scombrid fish, such as mackerel, contain high levels of the amino acid histidine (Nemetz and Shotts Jr, 1993). Decomposition of such fish produces significant amounts of histamine by bacterial decarboxylation of the histidine (Barancin *et al.*, 1998). Bacterial proliferation is likely to occur with any fish carcass left unrefrigerated for extended periods. Bacterial histamine contamination is an important source of fish poisoning when consumed by humans (Barancin *et al.*, 1998). However, there are no evidence of human health concerns when eating lobsters caught with mackerel as bait. If histamine is present in lobster baits, perchance it could adversely affect the health of harvested lobsters. Cases of mild poisoning in humans having consumed mackerel with high levels of histamine have been reported (Barancin *et al.*, 1998), but not from eating lobsters caught with mackerel. More recently, Castonguay *et al.* (1997) demonstrated the presence of small amounts of paralytic shellfish poisoning

(PSP) toxins in the Atlantic mackerel, although there are no cases of humans being poisoned by PSP toxins when eating lobster or mackerel. Perhaps the slight accumulation of toxins related to paralytic shellfish poisoning in mackerel may influence lobster health.

Using less care in the overall handling process of lobsters on board the boats is likely to result in loss of vigour. Tossing lobsters made them 2.9 times more likely to have loss of vigour at the processing plant than lobsters placed into the temporary storage unit, when the effect of other variables was controlled.

Groups of lobsters landed on rainy days were 6.3 times more likely to lose vigour compared to landings on days without rain. Lobsters are sensitive to fresh water exposure (Jury *et al.*, 1994; Ennis, 1995; McMahon, 1995), and heavy exposure to rain will likely be detrimental for lobsters, especially for prolonged exposure times.

The odds ratio for the maximum depth at which traps were set was 0.85 for each metre. For each 3 m of additional depth at which traps were set, the lobsters became approximately 1.6 times less likely to suffer from loss of vigour upon their arrival at processing plants. Colder water temperatures found at greater depths are perhaps closer to those preferred by lobster (Crossin *et al.*, 1998), and thus may have beneficial impacts on lobster vigour. Also, Lawton and Lavalli (1995) reported that lobsters may occasionally experience hypoxia in warm waters, especially in intertidal environments. Because of their aggressive and territorial behaviour (Aiken and Waddy, 1995), perhaps some lobsters were forced to move toward less optimal habitats.

Some correlation among the various variables in the GEE model was present. This explains the multicollinearity problems encountered during the model building process, especially among dummy variables. Multicollinearity concerns relationships among predictor variables, but does not directly involve the outcome (Kleinbaum *et al.*, 1988). GEE models do not take multicollinearity problems into account, which explains in part the disagreement in the significance of predictors and their impact among different models; after a predictor is included in a model, adding another predictor correlated to the previous one has relatively little to contribute and would then be non-significant. Care was taken during the model building process to avoid this situation.

GEE models are more efficient if variables are independent (Liang and Zeger, 1986). Predictors within fishing boats were clustered, but GEE models will deal with this problem; they allow for investigation of factors at up to two levels although in the absence of a within-cluster covariate, they still perform well (Pendergast *et al.*, 1996). No lobster-level predictors were kept in the final model.

Lobster health predictors at the group-level or boat-level would probably be more accessible to the industry, as they would not require individual assessments of lobsters. However, predictors at the individual or lobster-level should yield more accurate estimates of population health, assuming a valid sample. None of the lobster-level parameters assessed in this study proved to be good predictors of lobster health at the processing plant, as assessed by the vigour state. Other lobster level predictors, such as physiological parameters,

should be assessed to help define lobster health. The quantification of the Crustacean Hyperglycemic Hormone (CHH) being correlated with stress levels in some decapods including the American lobster, may have potential (Chang *et al.*, 1998; Paterson and Spanoghe, 1997). Inorganic ions, such as magnesium, calcium or potassium, metabolites and waste products like glucose concentration and ammonia levels in the haemolymph should also be considered for further research, as proposed by Paterson and Spanoghe (1997).

Perhaps the lack of correlation between the parameters used in this study and the vigour state of lobsters at the processing plants was due to the lack of statistical power, with only 35 lobsters with decreased vigour in the dataset. Although the internal validity of these results is acceptable, it may not be valid for lobster health and fishing practices outside of Prince Edward Island. The lack of correlation with the outcome may also be partly explained by the utilization of an outcome (that was vigour) not sensitive enough to detect lobster stress, or loss of liveliness. Epidemiological models using survival analysis methods to correlate lobster-level, boat-level, or transport-level parameters to lobster survival post-storage may be more informative, but are not appropriate for Prince Edward Island. Live lobster holding is rarely for more than 10-14 days in Prince Edward Island and therefore, the frequency of reduced lobster survival would probably not be sufficiently high to justify using survival models.

4.5 Conclusion

The main objective of this chapter was to establish an epidemiological model assessing the impact of different transportation, fishing and handling practices on the health and quality of lobsters upon arrival at processing plants. This objective was met, with four lobster health predictors identified, although no transportation condition factors were significant. The use of different baits influenced lobster vigour; mackerel appeared especially detrimental. Further investigations of the types and quality of bait used and its influence on lobster health should be conducted. Fishing practices using mackerel as bait should be discouraged. Lobster fishers should be encouraged not to toss lobsters; gentle handling enhances lobster vigour. Lobsters directly exposed to freshwater (i.e., rain) experienced vigour loss. Protection from these weather conditions would reduce industry losses. Setting lobster traps in deeper waters also appeared to be beneficial to lobster health, although this represents most definitely a practice that fishers cannot change; they must fish where lobsters can be found.

Although THP is used as a lobster health predictor by the industry, these data did not indicate that individual lobster vigour upon arrival at the processing plants was predicted by THP measurements. No association was found between low THP levels and decreased vigour at processing plants.

Lobsters caught in Prince Edward Island generally experience very low mortality rates pre-processing. Therefore, an examination of factors associated with survival is not feasible in Prince Edward Island. This low level of mortality is likely a result of several factors; most canner-sized lobsters (approximately 63.5-

81 mm) are held in pounds for extremely short periods and the majority of lobsters caught in Prince Edward Island waters are canner-sized lobsters. To examine handling factors on the boat and their association with survival, a similar evaluation should occur in other areas of Atlantic Canada where greater proportions of market-sized lobsters and also where greater proportions of weak lobsters are likely held for longer periods.

5. HEALTH ASSESSMENT AND ESTIMATED PREVALENCE

OF *ANOPHYROIDES HAEMOPHILA* AND *AEROCOCCUS VIRIDANS*

IN AMERICAN LOBSTERS (*HOMARUS AMERICANUS*) CAUGHT

IN THE WATERS OF PRINCE EDWARD ISLAND

5.1 Introduction

The lobster (*Homarus americanus*) fishery has been one of the most stable fisheries in Canada. While Canadian landings reached a record peak in 1991 with more than 48,000 metric tons (mt), in 1996 they were stable at 39,000 mt, with an estimated value of \$379 million (Can.) (Fisheries and Ocean Canada, 1999). To supply the market with live product year-round, the lobster industry maintains live lobsters in captivity for various periods. However, significant mortalities can occur during holding, resulting in tremendous financial losses. Mortalities pre-processing have been estimated by the industry to average 10-15% per year (Lobster Health Research Centre, 1999), which translates into annual losses of \$50-75 million (Can.).

Haemolymph parameters are used to define lobster health (Jussila *et al.*, 1997; MacKenzie *et al.*, 1997b). Paterson and Spanoghe (1997) suggested that haemolymph parameters such as total haemolymph protein (THP) could also be used as stress indicators in marine decapods. In any confinement situation such as a lobster pound, the likelihood of infectious disease outbreaks is probably higher than in a natural or wild environment, especially when many animals from various sources are kept together. Although many mortality problems can be

attributed to non-infectious causes, there are some infectious diseases among the possible causes for the mortalities during holding.

Gaffkemia, caused by the bacterium *Aerococcus viridans* var. *homari*, is probably the most important infectious disease of impounded lobsters with major economic impact (Martin and Hose, 1995), and should be considered to be an important potential cause of mortalities in live holding facilities. Due to the lack of exoenzymes, and thus lack of specific invasive powers, *A. viridans* requires the presence of open wounds or ruptured exoskeleton to infect lobsters (Stewart, 1975).

Ciliates are commonly found in invertebrates, including the edible crab (*Cancer pagurus*) (Bang *et al.*, 1972), the Dungeness crab (*C. magister*) (Armstrong *et al.*, 1981; Sparks *et al.*, 1982), marine isopods (Hibbits and Sparks, 1983), the Pacific oyster (*Crassostrea gigas*) (Bower and Meyer, 1993), and the American lobster (Sherburne and Bean, 1991; Cawthorn *et al.*, 1996).

Bumper car disease, or ciliate disease, is caused by the scuticociliate *Anophryoides haemophila* (Cawthorn *et al.*, 1996), and represents another potential cause of lobster mortality, especially during winter impoundment. This disease eventually leads to depletion of haemocytes, leading to significantly reduced clotting ability (Cawthorn, 1997). Lobsters can become infected by transmission of the ciliates through open wounds often inflicted during ecdysis, and possibly through the thin epithelium of the gills (Cawthorn, 1997).

A third disease of economic significance that affects lobsters in captivity is shell disease, also called rust disease, black spot or brown spot disease. Shell disease is an external infection thought to be caused by various opportunistic pathogens, resulting in degradation of the chitin component of the exoskeleton (Getchell, 1989).

Both gaffkemia and bumper car disease affect the wild population of lobsters in Atlantic Canada, with levels of *A. viridans* infected lobsters ranging from 0% up to 40% (Stewart *et al.*, 1966; Vachon *et al.*, 1981; Keith *et al.*, 1992), and levels of *An. haemophila* infection approaching 20% (Aiken *et al.*, 1973; Cawthron *et al.*, 1996). Shell disease appears to cause significant problems mostly in lobsters landed in the south-western part of Nova Scotia (Getchell, 1989).

5.2 Objectives

The objective of this study was to estimate the prevalence of the causative agent of bumper car disease, *An. haemophila*, and of the causative agent of gaffkemia, *A. viridans*, in freshly caught lobsters coming from the waters of Prince Edward Island, Canada. Shell disease was also subjectively estimated. A secondary objective of this study was to assess the association of the biological parameters total haemocyte counts (THC) and THP with the lobster overall physical condition using logistic regression models.

5.3 Materials and methods

5.3.1 Source of lobsters

Thirty-nine fishing ports on Prince Edward Island with more than 10 fishing boats per port actively fishing lobsters were identified in spring lobster fishing areas (LFA) 24, 26a and 26b (Figure 1). A random list of five ports was computer generated using a software (Minitab®, version 10.1, Minitab Incorporated, State College, Pennsylvania, USA, 1994). Visits to each selected port enabled investigators to identify two boats per port on which fishers were willing to participate in the study. An average of 12 market-sized lobsters (minimum carapace length of 81 mm) per boat for a total of 116 lobsters were purchased at the wharves directly from fishers on June 26-27, 1997. A similar protocol was carried out in the fall for LFA 25 (Figure 1), with an average purchase of 14 lobsters per boat for a total of 138 lobsters obtained directly from fishers on September 4-5, 1997. The overall number of lobsters sampled for both fishing seasons was 254 lobsters (see Table I).

5.3.2 Sampling

Lobster body weight recorded in kilograms, carapace length measured in millimetres from the caudal end of the eye socket to the caudal extremity of the dorsal carapace, sex, shell score (Appendix L), and overall physical condition index were recorded for each lobster. The overall physical condition index was a combination of a subjective assessment of the liveliness of the lobsters and their physical appearance; lobsters either had a normal physical condition index or a

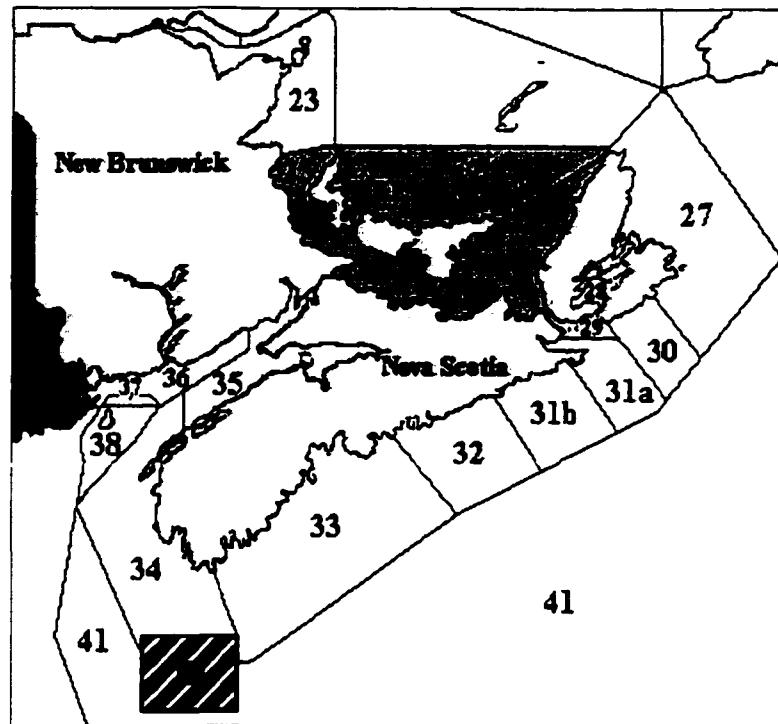


Figure 1. Schematic drawing representing the Lobster Fishing Areas (LFA) in Atlantic Canada. The spring sampling was conducted with 74 lobsters landed in LFA 24, 20 lobsters from LFA 26a and 22 lobsters landed in LFA 26b, while the fall sampling was carried out with 138 lobsters from LFA 25. Note that LFA 40 is closed to inshore and offshore lobster fishing. (DFO, 1998)

Table I. Summary results for the numbers of ports with corresponding lobster fishing areas (LFA), boats and lobsters sampled during the spring and fall seasons of 1997 in PEI.

FISHING PORT	LFA	SEASON	# OF LOBSTERS	# OF BOATS
1	24	spring	24	2
2	24	spring	26	2
3	26a	spring	20	2
4	24	spring	24	2
5	26b	spring	22	2
6	25	fall	28	2
7	25	fall	27	2
8	25	fall	27	2
9	25	fall	28	2
10	25	fall	28	2
TOTAL:			254	20

downgraded index. Lobsters were classified as downgraded, if they met at least one of the following criteria: dead, weak, open wound(s), active lesion(s), missing claw(s), missing leg(s), damaged claw(s), damaged leg(s), missing antenna(e), broken rostrum, damaged body or tail, or damaged antenna(e). Haemolymph was also sampled from every lobster for THP and THC measurements (see Appendix B for the haemolymph sampling protocol). The haemolymph sampling was conducted directly at the wharves.

Haemolymph was collected for isolation and identification of *A. viridans*, the causative agent of gaffkemia (Appendix B). Each lobster was euthanised with benzocaine (15 ml of stock solution/litre of saltwater; stock solution=100 g/L of ethanol). The left side of the carapace was cut open and removed, exposing the underlying tissues. Tissue samples of gills, hepatopancreas, tail muscle, heart and gonad were collected and fixed in a solution of 1G4F fixative (1% glutaraldehyde, 4% formaldehyde). Samples were submitted to the Lobster Health Research Centre (Charlottetown, Canada) for further indirect fluorescent testing using monoclonal antibodies (IFAT).

5.3.3 Statistical analysis

Data were transferred in a computer spreadsheet (Quattro® Pro version 7, Corel Corporation Limited, Ottawa, Ontario, Canada, 1996). A random sample of 60 records was examined and manually checked for data entry errors. Error checking for outliers and data description were conducted by examining

descriptive statistics including means, medians, standard deviations, minima and maxima for each continuous variable. The dataset was transferred into the statistical software STATA™ version 5.0 (Stata Corporation, College Station, Texas, USA, 1996) for further analysis which included Chi-square tests, T-tests, odds ratio calculations, least squares and logistic regressions. For all analyses, differences among groups were considered significant when $P<0.05$.

Unconditional associations between the outcome 'downgraded' and the potential predictors were estimated by Chi-square tests for the dichotomous variables (*A. viridans* and *A. haemophila*) and by T-tests for the continuous variables (body weight, carapace length, THC and THP). Parameters identified as possible predictors by these selection methods were kept for the logistic regression model building process. The regression models were built using stepwise, forward and backward selection procedures, with P -value for entry or removal of 0.05. The goodness-of-fit of the models was then assessed using the Hosmer-Lemeshow goodness-of-fit test, with the data divided into 10 groups. Standardized Pearson residuals were also examined.

5.4 Results

Every fisher approached in both sampling regimes agreed to cooperate, and therefore the rate of refusal to participate was zero. The monetary constraints and the difference in mean lobster weight resulted in different sample sizes for the two seasons.

5.4.1 Physical and physiological assessments

There were significantly more female lobsters assessed in the spring season than in the fall season, while the overall ratio was exactly one to one. Mean body weight, mean carapace length, THP and THC were significantly higher in the spring sample than in the fall. The overall averages were 0.51 kg for the weight, 86.6 mm for the CL, 51.9 g/L for the THP and the mean THC for both seasons was 25.8×10^6 haemocytes/ml (see Table II). However, no significant difference was observed in the proportion of downgraded lobsters between the spring and fall sampling, with an average of 13.8% of the sampled lobsters being downgraded. Figures 2 (a and b) show a positive association between THP and THC, in both males and females with r^2 of 38% and 9%.

Male lobsters had significantly higher THP levels than females in the spring (78.9 g/L compared to 66.9 g/L), but significantly lower in the fall (32.7 vs. 37.4 g/L) (see Table III). The THC values for males were also significantly higher than those of females in the spring (34.4×10^6 compared to 28.2×10^6 haemocytes/ml), but no statistically significant difference was found in the fall.

5.4.2 *Aeroccoccus viridans*, causative agent of gaffkemia

The prevalence of *A. viridans* infected lobsters was estimated to be 6.90% in the spring season, and 5.80% for the early part of the fall season (see Table IV). The overall prevalence of the causative agent of gaffkemia for both seasons combined was estimated at 6.30%. No significant difference in prevalence was found between the two seasons.

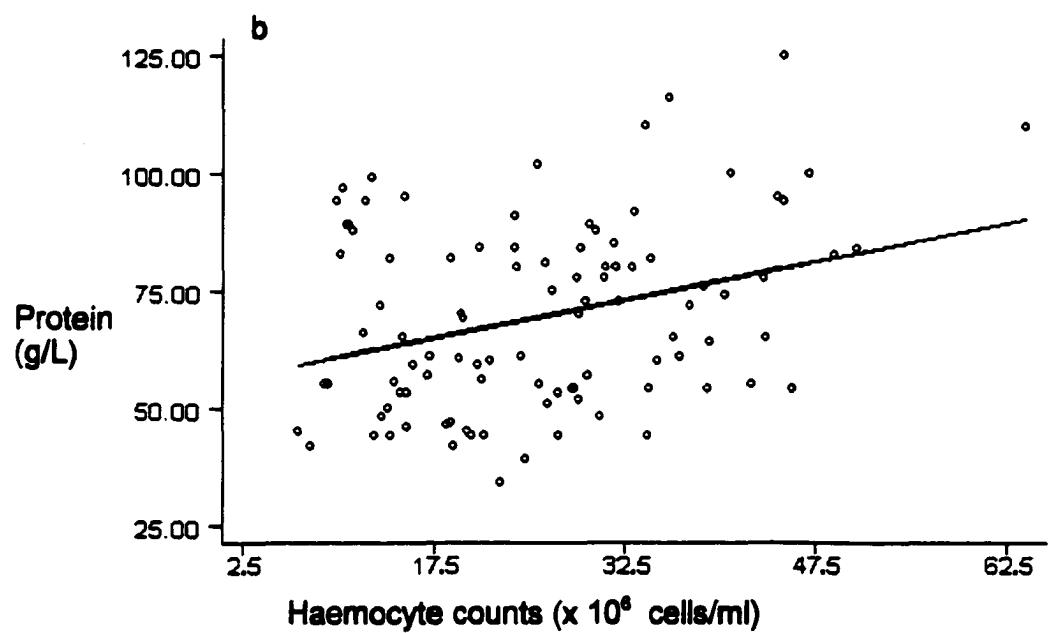
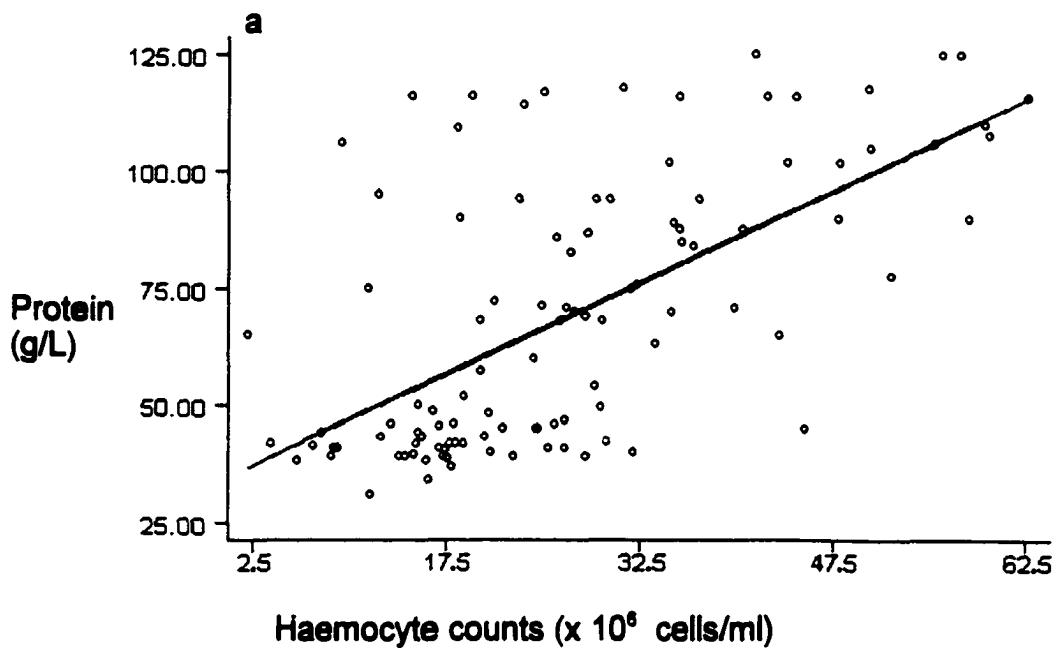
Table II. Summary results and mean values (standard deviation) of the physical and physiological parameters during the 1997 spring and fall lobster fishing sampling in PEI. Significant differences ($P \leq 0.05$) between the spring and fall seasons are represented by different superscripts.

PARAMETER	SAMPLING SEASON		
	SPRING	FALL	OVERALL
N	116	138	254
GENDER			
female	56.9%	44.1%	50.0%
male	43.1%	55.9%	50.0%
ratio female/male	1.32 ^a	0.79 ^b	1.00
BODY WEIGHT (kg)	0.58 ^a (0.2)	0.47 ^b (0.1)	0.51 (0.1)
CARAPACE LENGTH (mm)	88.9 ^a (7.8)	84.6 ^b (3.2)	86.6 (6.1)
THC ($\times 10^6$ haemocytes/ml)	30.1 ^a (13.7)	21.4 ^b (8.7)	25.8 (12.2)
THP (g/L)	72.0 ^a (17.4)	34.8 ^b (10.2)	51.9 (23.3)
DOWNGRADED (%)	12.9 (0.3)	14.5 (0.4)	13.8 (0.4)

N = number of observations

THC = total haemocyte counts

THP = total haemolymph protein



Figures 2 a & b. Relationship between total haemocyte counts and total haemolymph protein in male lobsters (a) ($r^2=0.38$, $P<0.001$), and in female lobsters (b) ($r^2=0.09$, $P<0.001$). Data were collected during the 1997 spring and fall seasons in PEI. Each data point represents one observation.
 H_0 : slope = 0.

Table III. Gender specific mean values (standard error) of the total haemolymph protein (THP) levels and total haemocyte counts (THC) in lobsters sampled during the 1997 spring and fall seasons in PEI. The t-statistic values with corresponding *P*-values were obtained when comparing male with female THC and THP levels.

PARAMETER	SEASON	MEAN VALUE (SE)		T-STATISTIC	<i>P</i> -VALUE
		MALE	FEMALE		
THP (g/L)	spring	78.9 (2.7)	66.9 (1.8)	3.94	0.0001
	fall	32.7 (1.2)	37.4 (1.2)	2.74	0.0070
THC ($\times 10^6$ haemocytes/ml)	spring	34.4 (2.1)	28.2 (1.6)	2.42	0.0172
	fall	20.2 (1.0)	22.8 (1.2)	1.65	0.1012

Table IV. Prevalence of *Aerococcus viridans*, shell disease and *Anophryoides haemophila* in lobsters sampled in the 1997 spring and in the fall seasons, freshly caught in PEI waters. No significant differences were found between seasons.

SEASON	N	CASES	A. VIRIDANS	AN. HAEMOPHILA	SHELL DISEASE
SPRING	116	# of positive	8	0	0
		Prevalence (95% CI)	6.90% (3.02%-13.14%)	0% (0%-3.13%) ¹	0% (0%-3.13%) ¹
FALL	138	# of positive	8	1	0
		Prevalence (95% CI)	5.80% (2.54%-11.10%)	0.72% (0.02%-3.97%)	0% (0%-2.64%) ¹
OVERALL	254	# of positive	16	1	0
		Prevalence (95% CI)	6.30% (3.64%-10.03%)	0.39% (0.01-2.17%)	0% (0%-1.44%) ¹

¹ one-sided 97.5% confidence interval

5.4.3 *Anophyoides haemophila*, causative agent of bumper car disease

Only one lobster was positive for the presence of *An. haemophila*, the causative agent of bumper car disease in the fall season, while no positives were observed in the spring season (see Table IV). Therefore, the prevalence in the spring was estimated at 0%, 0.72% in the fall, and 0.39% for both seasons combined. There were no statistical differences between sexes or seasons.

5.4.4 Shell disease

All of the lobsters assessed for external lesions of shell disease were given a score of '0' (both in the spring and the fall season). The estimated prevalence of shell disease for Prince Edward Island, for the spring and the fall season was therefore 0% (see Table IV).

5.4.5 Association with downgraded lobsters

The overall condition index, once dichotomized into 'downgraded' or 'not downgraded' (normal) was significantly associated with the THC and THP in the fall with lower values associated with downgraded lobsters, but not in the spring.

Tables VII and VIII show the results of the logistic regressions used to predict the overall condition (i.e., downgraded or not). THP and THC were correlated to each other, and only THC stayed as a significant predictor when forward and backward stepwise procedures were used, when both seasons were pooled, and in the fall season alone.

Table V. Mean values (SE) or proportions for the different parameters according to the overall condition of the lobsters assessed during the 1997 spring and fall seasons in PEI. *P*-values for comparisons between 'normal' and 'downgraded' were obtained by T-tests.

PARAMETER	SEASON	OVERALL CONDITION		<i>P</i> -VALUE
		NORMAL	DOWNGRADED ¹	
WEIGHT (kg)	spring	0.59 (0.02)	0.56 (0.09)	0.707
	fall	0.47 (0.01)	0.46 (0.01)	0.444
	overall	0.51 (0.01)	0.49 (0.03)	0.322
CARAPACE LENGTH (mm)	spring	89.2 (0.8)	87.0 (1.9)	0.310
	fall	84.5 (0.3)	85.0 (0.7)	0.564
	overall	86.7 (0.4)	85.8 (0.9)	0.457
TOTAL HAEMOLYMPH PROTEIN (g/L)	spring	72.6 (1.8)	68.6 (5.8)	0.405
	fall	35.7 (1.0)	29.2 (1.9)	0.008
	overall	52.8 (1.6)	46.1 (4.4)	0.114
TOTAL HAEMOCYTE COUNTS ($\times 10^6$ haemocytes/ml)	spring	31.0 (1.4)	30.3 (3.8)	0.839
	fall	22.7 (0.8)	11.3 (1.5)	0.001
	overall	26.5 (0.8)	20.8 (2.7)	0.017

¹ Downgraded lobsters consisted of lobsters with damaged or missing appendage(s), broken rostrum, and/or any other wound(s) or lesion(s).

Table VI. Proportion (frequency) of lobsters positive for *Anophryoides haemophila* and *Aerococcus viridans*, according to the overall condition of the lobsters assessed during the 1997 spring and fall seasons in PEI. *P*-values for comparisons between 'normal' and 'downgraded' were obtained by Chi-square tests.

PATHOGEN	SEASON	OVERALL CONDITION		EXACT <i>P</i> -VALUE (FISHER)
		NORMAL	DOWNGRADED ¹	
<i>AN. HAEMOPHILA</i> (IFAT) ²	spring	0% (0/101)	0% (0/15)	-
	fall	0% (0/118)	5.0% (1/20)	0.145
	overall	0% (0/219)	2.9% (1/35)	0.138
<i>A. VIRIDANS</i> (PEA broth + Gram staining) ³	spring	5.9% (6/101)	13.3% (2/15)	0.276
	fall	5.1% (6/118)	10.0% (2/20)	0.327
	overall	5.5% (12/219)	11.4% (4/35)	0.163

¹ Downgraded lobsters consisted of lobsters with damaged or missing appendage(s), broken rostrum, and/or any other wound(s) or lesion(s).

² Fluorescent testing methods using monoclonal antibodies (IFAT).

³ Culture on phenylethylalcohol broth (PEA broth), with confirmation by identification of Gram positive cocci under microscopic examination.

Table VII. Results of the logistic regression with 95% confidence interval to predict lobster downgrade, using a backward stepwise procedure with P -value for removal of 0.05. The variable *season* was forced into the model.

Log Likelihood = -62.075711	Number of obs = 190			
	Chi square (2) = 7.94			
	Prob > Chi square = 0.0189			
	Pseudo r^2 = 0.0601			
Downgraded	COEFFICIENT	Z	$P > z $	95% CONF. INTERVAL
SEASON	-0.683	-1.262	0.207	-1.743 0.377
THC	-0.007	-2.611	0.009	-0.011 -0.002
CONSTANT	0.551	0.456	0.649	-1.819 2.921

season = either spring or fall lobster fishing season in PEI

THC = total haemocyte counts

Table VIII. Results of the logistic regression with 95% confidence interval to predict lobster downgrade, using a backward stepwise procedure with P -value for removal of 0.05. The model was for the fall season only.

Log Likelihood = -29.168661	Number of obs = 127				
	Ch square (2) = 29.80				
	Prob > Chi square = 0.0000				
	Pseudo r^2 = 0.3381				
Downgraded	COEFFICIENT	Z	$P > Z $	95% CONF. INTERVAL	
THC	-0.027	-4.064	<0.001	-0.041	-0.014
CONSTANT	2.321	2.42	0.016	0.441	4.202

THC = total haemocyte counts

No parameters remained in the spring model, and therefore no spring models were developed. The coefficient for THC was approximately 4.2 times greater in the second model (i.e., fall only) than in the pooled model with season forced in, with a pseudo- r^2 of 33.8% versus 6.0% respectively, and there was no reason to assume that the models did not fit the data (Hosmer-Lemeshow test for pooled model yielded a Chi-square value of 12.9 and a corresponding P -value of 0.12, and a Chi-square value of 11.0 with a P -value of 0.20 for the 'fall' model). In both models, standardized Pearson residuals showed a mean of approximately zero (-0.01 and 0.01 for the pooled model and the fall model respectively) with a calculated variance close to one (1.08 and 0.98 for the pooled model and the fall model respectively). Therefore, the models fitted the data reasonably well.

5.5 Discussion

5.5.1 Physical and physiological assessments

In this study, THP and THC were two physiological parameters used to assess lobster health. The range for THP was 18-107 g/L, with 95% of the population included between 40.5 g/L and 63.2 g/L, while the range for THC was $2.2-64.0 \times 10^6$ haemocytes/ml, with 95% of the population comprised between 2.4×10^6 haemocytes/ml and 50.2×10^6 haemocytes/ml. MacKenzie *et al.* (1997b) reported a mean value for THC of 12.6×10^6 haemocytes/ml in their laboratory work, while Cornick and Stewart (1978) found slightly higher values of 18.1×10^6 haemocytes/ml. Although the two values are within the THC range reported in this study, they are somewhat lower than the mean values obtained

here, which were 30.9 and 21.3×10^6 haemocytes/ml in the spring and fall respectively. Both THC and THP in this study were obtained after the lobsters were landed on shore, and not as they were coming out of the water.

Jussila *et al.* (1997) suggested a stress reaction, from handling or exposure to air, could explain the high mean THC value that they found in western rock lobsters (*Panulirus cygnus*). Martin and Hose (1995) suggested that dehydration, different molting stages, nutritional or stress levels, could affect the THP and THC. These factors could all be possible explanations for the THP and THC values observed in this study. Perhaps the current study lobsters were more stressed than those described by MacKenzie *et al.* (1997b), but it is unlikely that the cellular response was sufficiently rapid to cause the higher THC.

No associations between THP or THC were observed with the prevalence of *A. viridans* or *An. haemophila* in this study. Hudson (1995) reported a THP of 75 g/L in one sand crab (*Portunus pelagicus*) infected with the dinoflagellate *Hematodinium australis*, while the average THP in uninfected crabs varied from 44.7 g/L to 57.1 g/L. This higher THP value for the infected crab contradicts the present results, as downgraded lobsters showed significantly lower THP levels than the normal ones. However, the 75 g/L value came from one crab only, and therefore, caution should be taken when drawing conclusions from the value reported by Hudson (1995).

Significant associations were found between THC and THP, and the overall condition index of lobsters in the fall; normal lobsters had higher levels than downgraded lobsters. This suggests that both THP and THC could be used

as potential indicators of lobster liveliness or physical condition, and possibly health. However, these relationships consisted of unconditional associations, and other variables, perhaps molting cycle or dehydration status, should be controlled in further studies. Previous findings (Chapter 4) suggested that neither THP nor THC were significant predictors for lobster vigour loss, while earlier work (Chapter 2) related THP to the lobster overall physical condition index, and appeared to be a potential stress indicator. Further studies assessing haemolymph parameters in controlled situations are needed for a better understanding of their relationships with lobster health.

When building a model to predict lobster downgrades, only THC remained as a significant predictor in the fall model and in the model including the season variable. In the two models, higher THC was associated with better lobster condition. In the fall model, THC alone could explain 33.8% of the variance in the data, compared to 6% when both seasons were combined. This substantial difference between the two pseudo- r^2 values can easily be explained by the significant difference between the mean values of THC in the spring versus the fall. The quality of outcome variable, downgraded or not, may limit the external validity of these results; having a reliable and objective way to determine lobster health would tremendously increase the significance of these data. Nevertheless, these data clearly shows that THC is related to a lobster's physical condition and could subsequently be used as an indicator (and perhaps a predictor) of lobster health, as suggested by Patterson and Spanoghe (1997), Jussila *et al.* (1997), and MacKenzie *et al.* (1997b).

5.5.2 Pathogen prevalence

5.5.2.1 *Aerococcus viridans*, agent of gaffkemia

Gaffkemia is a disease endemic to lobster populations of North America, and has also been reported in European waters (Alderman, 1996). Huang and Bayer (1989) reported *A. viridans* prevalence of 6.7% in freshly caught lobsters off the coast of Maine. The prevalence of *A. viridans* (formerly identified as *Gaffkya homari*) in Atlantic Canada was estimated at almost 5% by Stewart *et al.* (1966), while site specific levels ranged from 0 to 22% according to Vachon *et al.* (1981). Keith *et al.* (1992) reported that 0 to 40% of lobsters caught in Canadian locations were infected with *A. viridans*.

Both spring and fall sampling were done over two consecutive days, and may not reflect the true prevalence during the two fishing seasons. However, this sampling protocol was necessary to get a point estimate of prevalence that would not be affected by changing water temperature over a longer period. The overall prevalence of *A. viridans* infected lobsters freshly caught out of the waters of Prince Edward Island for the summer and fall fishing seasons was 6.3% in 1997.

Lobsters cannot control or efficiently respond to infection with *A. viridans* (Stewart and Zwicker, 1974; Marks *et al.*, 1992). In *A. viridans* infected lobster, there is no agglutination and the bactericidal ability of the haemolymph deteriorates in the presence of virulent strains of *A. viridans* (Stewart, 1975; Johnson *et al.*, 1981). The only factor which appears to affect the prevalence of

gaffkemia in *A. viridans* infected lobsters is the water temperature; a decreased mean time to death with increasing water temperatures would result in increased gaffkemia incidence (Stewart, 1975; Bayer and Daniel, 1987) and mortality. Lobsters infected with *A. viridans* will eventually die of gaffkemia, which explains some of the confusion in the literature when reporting prevalence of *A. viridans* versus prevalence or even incidence of gaffkemia. Nevertheless, these infection levels agree with the prevalence found in this study.

5.5.2.2 *Anophryoides haemophila*, agent of bumper car disease

In the fall, only one lobster was positive for the presence of the ciliate *An. haemophila*. In the spring, no lobsters infected with *An. haemophila* were observed. These results were surprising, because earlier studies showed up to 17.8% prevalence of *An. haemophila* in lobsters caught in Prince Edward Island waters (Cawthorn *et al.*, 1996), and up to 20% in New Brunswick (Aiken *et al.*, 1973). The most likely explanation was that the true prevalence was low, and that the lobster population sampled was different from those reported by Aiken *et al.* (1973) and Cawthorn *et al.* (1996). Low prevalence could also reflect environmental factors, such as increasing sea water temperatures, or a seasonal cycle of *An. haemophila* in the wild. In the present study, the tissue samples were collected from fresh lobsters and then fixed before being analysed. Fixing the lobsters whole and then collecting the appropriate samples could be an alternative.

5.5.2.3 Shell disease

No evidence of shell disease was found during this study. The epidemiology and the aetiology of shell disease is still poorly understood (Prince *et al.*, 1993b). Although it is a significant problem in lobster holding facilities throughout the Canadian Maritime provinces and the state of Maine, USA, shell disease appears to be restricted mostly to lobsters landed in southwest Nova Scotia, and to a lesser degree from the Jonesport area in Maine (Getchell, 1989). This disease is seen almost exclusively during the winter months and early spring. A multi-factorial aetiology is proposed for the disease (Prince *et al.*, 1993a). One major component responsible for the onset of shell disease could be rough handling of the animals in a cold environment on board fishing boats, such as the weather of southwest Nova Scotia in November, December, and January. The prevalence of shell disease in lobsters landed in Prince Edward Island waters, from June to October was expected to be extremely low, since cases of shell disease have rarely been reported. The absence of shell disease in lobsters examined during this study was not surprising.

5.6 Conclusion

The primary objectives of estimating the prevalence of *An. haemophila* and *A. viridans* was achieved, and so was the secondary objective regarding the examination of possible associations between THP and THC with lobster overall physical condition index.

The prevalence of *An. haemophila*, the causative agent of bumper car disease, was extremely low, and most likely reflected a different population of sampled lobsters compared to previous studies (Aiken *et al.*, 1973; Cawthorn *et al.*, 1996), or a possible environmental influence on the seasonal cycle of the wild parasites. *A. viridans*, the causative agent of gaffkemia, was found in levels within the range of previous studies reporting prevalence (Stewart *et al.*, 1966; Vachon *et al.*, 1981; Keith *et al.*, 1992). Because more than 6% of the lobsters examined were positive for *A. viridans*, the industry should strongly consider testing for this lethal pathogen before stocking their holding facilities, especially when water temperatures are elevated.

In this study, THP and THC were two physiological parameters used to assess lobster health. Significant correlations were found between THC and the lobster overall condition index in the fall, and also between THP and the overall condition index. For both THP and THC, normal lobsters had on average higher levels than downgraded lobsters. Both THP and THC could be used as potential predictors or indices for lobster liveliness and physical condition, and therefore health.

The measurement of THP can be easily achieved in the field via the refractometer technique (MacKenzie *et al.*, 1997a), and could represent a useful industrial tool for assessing lobster health. Although THC appeared more correlated to lobster physical condition index, it is time consuming, requires special buffer and equipment, and thus would be less likely to be utilized in the

field as a lobster health predictor. The results showed a significant difference between male and female lobsters for THP and THC, and therefore, care must be taken when assessing haemocyte counts and total protein compared to THP. Further research is required to fully understand the interaction of gender with THP or THC in the American lobster.

6. GENERAL DISCUSSION

6.1 Introduction

Because of the importance of the lobster industry in Atlantic Canada, any improvement in the quality of the post-harvest sector of this fishery should yield tremendous economic results. The work achieved through this thesis certainly represents an important step toward a better product prior to processing.

6.2 Lobster quality variation

When physical condition was assessed in freshly landed lobsters, there was significant variations in the quality and health of lobsters among different wharves. This degree of variation in lobster health was expected as these assessments were carried out on lobsters coming from different geographic regions, at different times of the year, with different water temperatures, and most likely where different sources of food and prey were available. Many of these factors are known to affect plasma protein, molting cycle, and the overall health status (Ennis, 1973; Paterson and Spanoghe, 1997).

Significant differences in overall physical condition index and total haemolymph protein (THP) levels were demonstrated among fishing boats from common wharves. If the weather and environmental conditions were similar among boats fishing from common wharves but yet significant variations in lobster health were present, fishing practices themselves were likely to have considerable impacts on lobster health.

6.3 Impact of fishing practices

When fishing practices were monitored on board Prince Edward Island boats in 1997, and their impacts on lobster health assessed, approximately 20 different practices recorded on the boats and during transport from the wharves to the processing plants had significant unconditional associations (direct association without controlling for possible confounder or interaction) with lobster quality. When the effect of all of these possible predictors was analysed in a Generalized Estimating Equation logistic regression model (GEE), four predictors were retained. The significant predictors for loss of vigour at arrival at the processing plant were: use of mackerel for bait, possibility of exposure to rain, and rough handling of lobsters on the boat. The maximum depth at which traps were set had a protective effect with increasing depth. However, there is definitely a need for further studies addressing the problem of multicollinearity between determinants, the lack of transportation factors that were identified in the current models, and also there is a need for further studies using a reliable and objective outcome.

When lobsters were landed on rainy days, the chance of observing decreased vigour at arrival at processing plants was more than 10 times higher than if it did not rain. Lobsters landed from boats using mackerel bait were more than 7 times more likely to suffer from loss of vigour when arriving at processing plants than lobsters fished with any other kind of bait. Lobsters were 3.6 times more likely to have loss of vigour if handled roughly compared to being handled gently on board fishing vessels. Setting the fishing traps in deeper waters had a

protective effect on lobster health: for every 3 metre increment in the depth, lobsters were 1.6 times less likely to suffer from a loss of vigour. Protection from exposure to freshwater should be a priority on every fishing boat. The potential impact of lobster bait and bait storage characteristics on overall health should be further investigated.

6.4 Pathogen prevalence

Gaffkemia, is probably one of the most important infectious diseases of impounded lobsters (Martin and Hose, 1995). Bumper car disease, or ciliate disease also represents a potential lobster killer, especially in the winter impoundment period (Cawthorn *et al.*, 1996). When certain stocks of lobsters are impounded during winter, shell disease may develop and seriously impair productivity due to the aesthetic appearance of affected lobsters, particularly for lobsters from LFA 33 & 34 (Getchell, 1989).

The prevalence of *Anophryoides haemophila*, the causative agent of bumper car disease was lower than infection levels previously reported (Aiken *et al.*, 1973; Cawthorn *et al.*, 1996), and may have reflected different lobster populations than in other studies. Seasonal cycle of the parasites in the wild may also account for the low prevalence. *Aerococcus viridans*, the causative agent of gaffkemia, was detected at levels similar to other studies (Stewart *et al.*, 1966; Vachon *et al.*, 1981; Keith *et al.*, 1992). The prevalence of infection with *A. viridans*, was up to 11.3% in the fall, and therefore the industry should

seriously consider the influence of this pathogen on survival of lobsters maintained in holding systems, especially during months when water temperatures are elevated. Shell disease was not observed.

6.5 Health predictors

Total haemocyte counts (THC) and THP were two physiological parameters used to assess lobster health. No association was found between low THP levels and mortality rates. On average, normal lobsters had significantly higher THP and THC levels than downgraded lobsters. This indicates that both THP and THC could be promising predictors or indices for lobster liveliness, physical condition, and possibly health. THP levels vary greatly according to the time of year and gender. Such factors should always be taken into consideration when using total protein as an indicator of health. THC may be more reliable health indicators than THP, if the influence of the dehydration could be controlled. However, measuring THC is time consuming and requires special buffer and equipment. Thus, THC would be less useful in the field as lobster health predictors. Both THP and THC were related to lobster vigour or lobster physical condition index when assessed with unconditional association. Factors that influence THP and THC levels need to be much better understood and quantified, before they become useful indicators or predictors of lobster health.

6.6 Dehydration

The difference in THC from boats to processing plants suggested haemoconcentration. This was evidenced by the increase in the number of cells per millimetre of haemolymph (due to the dehydration status of the lobsters through time). This phenomenon makes comparisons among groups of lobsters difficult to interpret. Further studies addressing the impact and quantification of dehydration are required. Newsome *et al.* (1994) suggested that spraying red swamp crawfish (*Procambarus clarkii*) with seawater could protect them against dehydration through evaporation during transport. This avenue should be investigated for transport of lobsters, even for short distances.

6.7 Gender differences

Gender is a classic example of a host parameter being a confounding variable (Martin *et al.*, 1987), and therefore some degree of gender influence on lobster health indicators should be expected. The results showed significant differences between male and female lobsters for body weight, carapace length, and health parameters such as overall physical condition index, THP and THC. Cautious interpretation is necessary when assessing lobster health, particularly when THC and THP are being measured. Male lobsters caught in Prince Edward Island waters in the spring showed significantly higher THP levels than females, but significantly lower in the fall. However, this difference was not always present when the lobsters were coming from other regions of the

Maritime provinces. Cornick and Stewart (1978) did not find any gender difference when they performed differential haemocyte counts in the American lobster, nor did Jussila *et al.* (1997) when assessing haemocyte counts on the rock lobster. MacKenzie *et al.* (1997a) only reported a weak difference between male and female lobster THP in their laboratory work, while Chen and Chia (1997) reported no difference at all in THP levels between male and female mud crabs (*Scylla serrata*). Further research is required to fully understand the interaction of gender and physiological parameters in the American lobster, perhaps through the design of matching trials, or gender specific or excluding studies.

6.8 Study objectives

The major objectives for the research described in this thesis were to quantify lobster industry losses occurring during holding, to assess factors (i.e., lobster fishing and handling practices, transportation conditions, and lobster health assessment indices) contributing to these losses, and to identify where the losses are occurring in the post-harvest industry. Another important objective was to estimate the prevalence of the major lobster infectious diseases affecting the post-harvest industry, and their association with lobster health and quality. All of the objectives were indeed accomplished to some degree during these studies.

In conclusion, due to the nature of this industry, keeping groups of lobsters identified throughout the entire 'production' cycle was extremely difficult

to achieve, especially in a tidal pound environment. Tagging and following lobsters from the fishing wharves to the holding facilities enabled the investigators to appreciate the complexity and sometimes the capriciousness of the industry. Nevertheless, the work accomplished and presented in this thesis represents a solid base and advancement into improving the quality of the lobster industry in Atlantic Canada.

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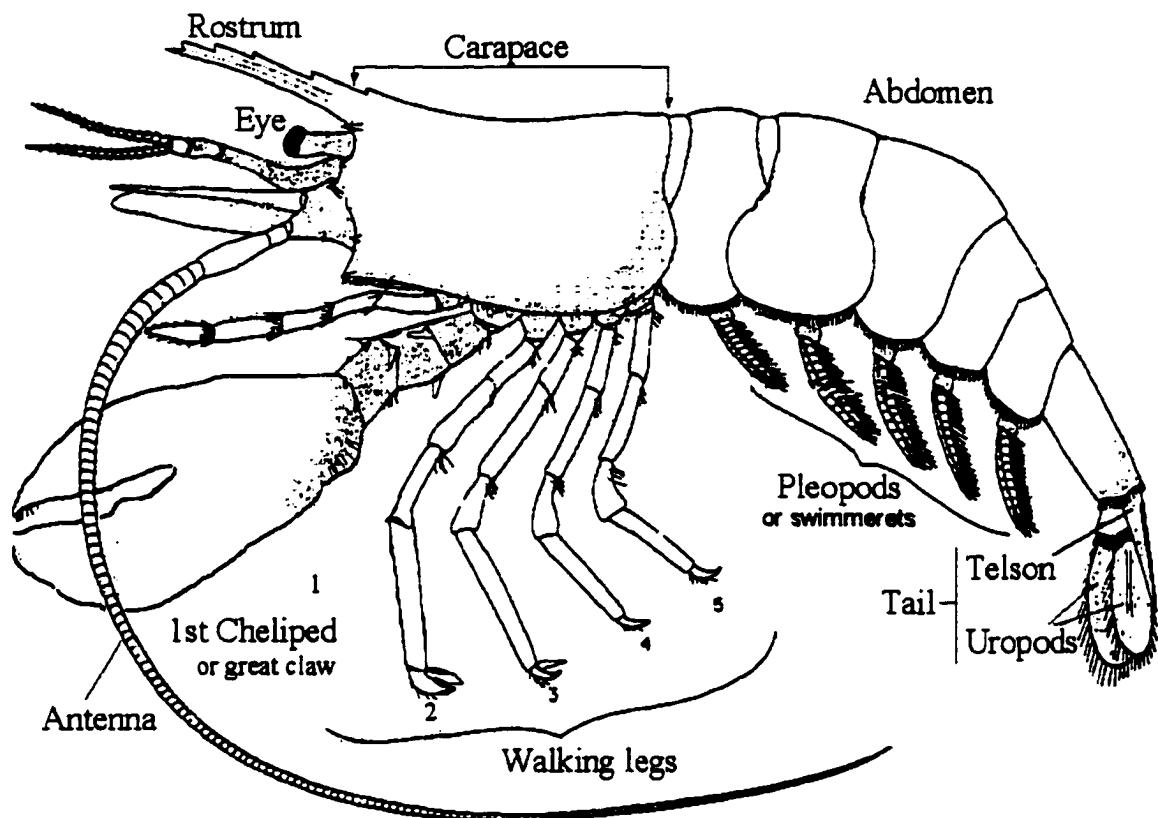
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Appendix A. External anatomy of the adult American lobster, *Homarus americanus*.



Appendix B. Haemolymph sampling procedures.

Bleeding

Using a 3 ml syringe with a 23-gauge needle, 1.6 ml of haemolymph was aseptically removed from the ventral sinus after swabbing the ventral surface with 70% alcohol.

Total haemocyte counts (THC)

THC were performed according to the method described by Mackenzie *et al.* (1997b). Haemolymph (0.5 ml) was added to a 10 ml plastic tube containing 4.5 ml of artificial sea water (ASW) buffer with 0.1% formalin. The tubes were inverted 20-30 times and placed on ice until further microscopic examination. Total haemocyte counts were performed using a haemocytometer (double dark line Neubauer improved counting chamber, la Fontaine, Dynatech, Germany) under light microscopy (Zeiss Standard 16 microscope, Germany) with a 40 power objective, by counting 20 squares per sample.

Aerococcus viridans isolation

The presumptive phenylethylalcohol (PEA) broth test was used for *A. viridans* isolation (Stewart *et al.*, 1966). Haemolymph (0.5 ml) was added to 4.5 ml of PEA broth, vigourously shaken and placed in an incubator at 28 °C for 96 h. Duplicate PEA broth were inoculated for each lobster. Suspicious broth culture tubes were identified by the typical purple to green to yellow color change of the broth, and confirmatory testing for the presence of *A. viridans* was performed using Gram stain and microscopic examination (Zeiss Standard 16 microscope, Germany).

Total haemolymph protein (THP)

Haemolymph (0.1 ml) was placed on a temperature compensated refractometer for direct reading of total haemolymph protein. The reading was then incorporated in the following formula (Mackenzie *et al.*, 1997a):

$$\text{Lobster haemolymph protein} = 0.95 \text{ (refractometer reading)} - 11.9$$

Appendix C. Comparison of the gender ratios in the different lobster sampling groups. The absolute values of the difference in the proportion of males between the row and the column are presented with corresponding exact *P*-values in parentheses (Chi-square done on individual combination). For example, the mean proportion of males in group 1 (~75%) - the proportion of males in group 2 (~44%) = ~31% (or 0.311), the difference between the two means being significant (*P*=0.002). * indicates a *P*-value <0.001.

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GROUPS	1	2	3	4	5	6	7	8	9	10
2	0.311 (0.000)*	-	-	-	-	-	-	-	-	-
3	0.043 (0.520)	0.268 (0.017)	-	-	-	-	-	-	-	-
4	0.410 (0.000)*	0.099 (1.000)	0.367 (0.000)*	-	-	-	-	-	-	-
5	0.285 (0.002)	0.026 (1.000)	0.242 (0.419)	0.125 (1.000)	-	-	-	-	-	-
6	0.321 (0.000)*	0.011 (1.000)	0.279 (0.008)	0.088 (1.000)	0.037 (1.000)	-	-	-	-	-
7	0.227 (0.000)*	0.084 (1.000)	0.184 (0.001)	0.183 (0.001)	0.058 (1.000)	0.095 (1.000)	-	-	-	-
8	0.250 (0.000)*	0.061 (1.000)	0.207 (0.012)	0.160 (0.258)	0.035 (1.000)	0.071 (1.000)	0.023 (1.000)	-	-	-
9	0.241 (0.001)	0.070 (1.000)	0.198 (0.015)	0.169 (0.119)	0.044 (1.000)	0.080 (1.000)	0.015 (1.000)	0.009 (1.000)	-	-
10	0.244 (0.000)*	0.067 (1.000)	0.201 (0.000)*	0.166 (0.102)	0.041 (1.000)	0.078 (1.000)	0.017 (1.000)	0.006 (1.000)	0.003 (1.000)	-
11	0.206 (0.000)*	0.105 (1.000)	0.163 (0.204)	0.204 (0.001)	0.079 (1.000)	0.116 (1.000)	0.021 (1.000)	0.044 (1.000)	0.035 (1.000)	0.038 (1.000)

Appendix D. Comparison of the weights (kg) before impoundment in the different lobster sampling groups. The absolute values of the difference in the mean lobster weight between the row and the column are presented with corresponding *P*-values in parentheses (one way ANOVA with Bonferroni correction). * indicates a *P*-value <0.001.

GROUP	2	3	4	5	6	7	9	10
3	0.311 (0.489)	-	-	-	-	-	-	-
4	0.032 (1.000)	0.279 (0.239)	-	-	-	-	-	-
5	0.888 (0.000)*	0.577 (0.001)	0.856 (0.000)*	-	-	-	-	-
6	0.344 (0.388)	0.033 (1.000)	0.312 (0.283)	0.543 (0.006)	-	-	-	-
7	0.082 (1.000)	0.393 (0.075)	0.114 (1.000)	0.970 (0.000)*	0.423 (0.065)	-	-	-
9	0.108 (1.000)	0.420 (0.000)*	0.140 (1.000)	0.996 (0.000)*	0.453 (0.000)	0.027 (1.000)	-	-
10	0.370 (0.015)	0.059 (1.000)	0.338 (0.001)	0.518 (0.000)*	0.025 (1.000)	0.452 (0.001)	0.478 (0.000)*	-
11	0.525 (0.000)*	0.251 (0.424)	0.793 (0.000)*	0.363 (0.086)	0.180 (1.000)	0.607 (0.000)*	0.633 (0.000)*	0.155 (0.035)

Appendix E. Comparison of the carapace lengths (mm) before impoundment in the different lobster sampling groups. The absolute values of the difference in the mean carapace length between the row and the column are presented with corresponding *P*-values in parentheses (one way ANOVA with Bonferroni correction). * indicates a *P*-value <0.001.

GROUP	1	2	3	4	5	6	7	9	10
2	5.1 (0.468)	-	-	-	-	-	-	-	-
3	0.6 (1.000)	5.7 (0.161)	-	-	-	-	-	-	-
4	8.1 (0.001)	3.0 (1.000)	8.7 (0.000)*	-	-	-	-	-	-
5	4.8 (1.000)	9.8 (0.003)	4.2 (1.000)	12.8 (0.000)*	-	-	-	-	-
6	2.0 (1.000)	3.1 (1.000)	2.6 (1.000)	6.1 (0.123)	6.7 (0.341)	-	-	-	-
7	8.7 (0.006)	3.6 (1.000)	9.3 (0.001)	0.6 (1.000)	13.5 (0.000)*	6.8 (0.208)	-	-	-
9	8.8 (0.002)	3.7 (1.000)	9.4 (0.001)	0.7 (1.000)	13.5 (0.000)*	6.8 (0.133)	0.1 (1.000)	-	-
10	0.6 (1.000)	4.5 (1.000)	1.2 (1.000)	7.5 (0.008)	5.4 (1.000)	1.3 (1.000)	8.1 (0.024)	8.2 (0.012)	-
11	1.2 (1.000)	6.3 (1.000)	0.6 (1.000)	9.3 (0.035)	3.6 (1.000)	3.2 (1.000)	9.9 (0.048)	10.0 (0.033)	1.8 (1.000)

Appendix F. Comparison of the overall physical condition before impoundment in the different lobster sampling groups. The absolute values of the difference in the proportion of lobsters with normal physical condition between the row and the column are presented with corresponding exact *P*-values in parentheses (Chi-square done on individual combination). * indicates a *P*-value <0.001.

GROUP	1	2	3	4	5	6	7	8	9	10
181	2	0.12 (0.011)	-	-	-	-	-	-	-	-
	3	0.06 (0.266)	0.05 (0.230)	-	-	-	-	-	-	-
	4	0.04 (1.000)	0.08 (0.091)	0.02 (0.644)	-	-	-	-	-	-
	5	0.01 (1.000)	0.12 (0.032)	0.07 (1.000)	0.05 (0.586)	-	-	-	-	-
	6	0.01 (1.000)	0.12 (0.012)	0.07 (0.229)	0.04 (0.489)	0.01 (1.000)	-	-	-	-
	7	0.16 (0.011)	0.28 (0.000)*	0.22 (0.000)*	0.20 (0.001)	0.15 (0.057)	0.16 (0.023)	-	-	-
	8	0.05 (0.375)	0.16 (0.000)*	0.11 (0.012)	0.09 (0.055)	0.04 (0.688)	0.04 (0.510)	0.11 (0.025)	-	-
	9	0.06 (0.258)	0.17 (0.000)*	0.12 (0.005)	0.09 (0.035)	0.05 (0.560)	0.05 (0.426)	0.10 (0.029)	0.01 (0.867)	-
	10	0.06 (0.060)	0.05 (0.141)	0.01 (0.854)	0.03 (0.454)	0.07 (0.173)	0.07 (0.083)	0.22 (0.000)*	0.11 (0.000)*	0.12 (0.000)*
	11	0.04 (0.545)	0.15 (0.000)*	0.10 (0.023)	0.07 (0.092)	0.03 (0.837)	0.03 (0.616)	0.12 (0.014)	0.01 (0.712)	0.02 (0.496)
										0.10 (0.000)*

Appendix G. Comparison of total haemolymph protein levels (g/L) before impoundment in the different lobster sampling groups. The absolute values of the difference in the mean THP levels between the row and the column are presented with corresponding *P*-values in parentheses (one way ANOVA with Bonferroni correction). * indicates a *P*-value <0.001.

GROUP	1	2	3	4	5	6	7	9	10
2	41.3 (0.000)	-	-	-	-	-	-	-	-
3	0.9 (1.000)	42.1 (0.000)*	-	-	-	-	-	-	-
4	37.8 (0.000)*	3.5 (1.000)	38.6 (0.000)*	-	-	-	-	-	-
5	29.5 (0.000)*	11.8 (0.021)	30.3 (0.000)*	8.3 (0.501)	-	-	-	-	-
6	13.4 (0.000)*	27.9 (0.000)*	14.2 (0.000)*	24.4 (0.000)*	16.1 (0.000)*	-	-	-	-
7	86.0 (0.000)*	44.7 (0.000)*	86.8 (0.000)*	48.2 (0.000)*	56.5 (0.000)*	72.6 (0.000)*	-	-	-
9	65.1 (0.000)*	23.9 (0.000)*	66.0 (0.000)*	27.3 (0.000)*	35.7 (0.000)*	51.8 (0.000)*	20.9 (0.000)*	-	-
10	37.0 (0.000)*	4.3 (1.000)	37.9 (0.000)*	0.8 (1.000)	7.5 (1.000)	23.6 (0.000)*	49.0 (0.000)*	28.1 (0.000)*	-
11	28.4 (0.000)*	12.9 (0.041)	29.2 (0.000)*	9.4 (0.606)	1.1 (1.000)	15.0 (0.007)	57.6 (0.000)*	36.7 (0.000)*	8.6 (1.000)

Appendix H. Comparison of the *Aerococcus viridans* prevalence (%) before impoundment in the different lobster sampling groups. The absolute values of the difference in the proportion of lobsters positive for gaffkemia between the row and the column are presented with corresponding exact *P*-values in parentheses (Chi-square done on individual combination). * indicates a *P*-value <0.001.

GROUP	5	7	9	10
7	0.00 (1.000)	-	-	-
9	0.00 (1.000)	0.00 (1.000)	-	-
10	0.08 (0.085)	0.08 (0.080)	0.08 (0.036)	-
11	0.24 (0.001)	0.24 (0.000)*	0.24 (0.000)*	0.16 (0.041)

Appendix I. Comparison of the weights (kg) after impoundment in the different lobster sampling groups. The absolute values of the difference in the mean lobster weight between the row and the column are presented with corresponding *P*-values in parentheses (one way ANOVA with Bonferroni correction). * indicates a *P*-value <0.001.

GROUP	7	8	9
8	0.005 (1.000)	-	-
9	0.021 (1.000)	0.016 (1.000)	-
10	0.493 (0.000)*	0.498 (0.000)*	0.514 (0.000)*

Appendix J. Comparison of the overall physical condition after impoundment in the different lobster sampling groups. The absolute values of the difference in the proportion of lobsters with normal physical condition between the row and the column are presented with corresponding exact *P*-values in parentheses (Chi-square done on individual combination). * indicates a *P*-value <0.001.

GROUP	5	7	8	9
7	0.22 (0.000)*			
8	0.81 (0.000)*	0.59 (0.000)*	-	-
9	0.48 (0.000)*	0.26 (0.000)*	0.33 (0.000)*	-
10	0.47 (0.000)*	0.26 (0.000)*	0.34 (0.000)*	0.01 (1.000)

Appendix K. Comparison of total haemolymph protein levels (g/L) after impoundment in the different lobster holding pounds. The absolute values of the difference in the mean THP levels between the row and the column are presented with corresponding *P*-values in parentheses (one way ANOVA with Bonferroni correction). * indicates a *P*-value <0.001.

GROUP	7	8	9
8	15.5 (0.000)*	-	-
9	1.4 (1.000)	14.1 (0.000)*	-
10	14.7 (0.000)*	0.8 (1.000)	13.3 (0.000)*

Appendix L. Scoring key used for the description of shell disease in American lobster.

SHELL SCORE	DESCRIPTION
0	no lesions
1	small area (less than one cm diameter), superficial lesion
2	large area (more than one cm diameter) or multiple lesions, superficial lesion
3	small area (less than one cm diameter), deep lesion
4	large area (more than one cm diameter) or multiple lesions, deep lesion
5	perforated shell

Appendix M. Handling and fishing practices questionnaire.



LOBSTER HEALTH RESEARCH CENTRE

University of Prince Edward Island
550 University Avenue,
Charlottetown, PE
C1A 4P3, Canada

Tel.: (902) 566-0584
Fax: (902) 566-0851

Our file number: _____

Fishing Handling Assessment

Survability of Prince Edward Island Lobsters

X

A) IDENTIFICATION AND BOAT SPECIFICATIONS

1. **Name of the boat:** _____
2. **Vessel number (CFV):** _____
3. **Owner's name (s):** _____
- 3.1 **Address:** _____

- 3.2 **Tel.:** (____) _____ **Fax:** (____) _____
4. **Crew size:** _____ people total on that day (excluding yourself)
5. **Boat size:** _____ feet
6. **Years fishing (owner):** _____ years
7. **Name of the Wharf:** _____
7.1 **Contact person:** _____
Tel.: (____) _____ **Fax:** (____) _____
8. **Name of the buyer (Company) at the wharf:** _____
8.1 **Contact person:** _____
Tel.: (____) _____ **Fax:** (____) _____
9. **Name of the holding facility/processing plant where the lobsters are being shipped**

- 9.1 **Contact person:** _____
Tel.: (____) _____ **Fax:** (____) _____

B) ENVIRONMENT

1. *Air temperature*

1.1 *Max.:* _____ °C 1.2 *Min.:* _____ °C

2. *Wind* (Check ONE)

None Light
 Moderate Strong
 Storm

3. *Rain*

3.1 *Strength* (Check ONE)

None Light
 Moderate Heavy
 Storm Mist

3.1.1 *If it did rain, how long was it for?* (Check ONE)

Less than 2 hours 2-4 hours
 4-6 hours More than 6 hours

3.1.2 *Was it:* (Check ONE)

Continuous Intermittent

4. *Sunshine* (Check ONE)

None Less than 2 hours
 2-4 hours 4-6 hours
 More than 6 hours

5. *Water temperature:*

_____ °C

6. *Waves* (Check ONE)

None Less than 2 feet
 2-4 feet 4-6 feet
 More than 6 feet

7. *Depth of traps*

7.1 *Max.:* _____ feet

7.2 *Min.:* _____ feet

C) FISHING PRACTICES

1. *Trap setting* (Please give an estimated % if more than one type of setting is used)

____% Single ____% Double

____% Multiple, specify how many traps per line: _____

2. *Hauling system*

() **Hydraulic system** () **Manually**
() **Other:** _____

2.1 *If using a hydraulic system, is the next trap hanging in the air while the first one is being empty?*

() **YES** () **NO**

2.2 *On which side of the boat are the traps retrieved?*

() **Right-Front** () **Left-Front** () **Left-Middle**
() **Right-Back** () **Left-Back** () **Right-Middle**

3. *Type of bait (used the previous day)*

Please, specify: _____

4. *Are the lobsters banded?*

4.1 CANNER () **YES** () **NO**

4.2 MARKET () **YES** () **NO**

4.3 *If YES, is it in both claws?*

4.3.1 CANNER () **YES** () **NO**

4.3.2 MARKET () **YES** () **NO**

5. *Grading procedures on the boat*

5.1 *Contact among lobsters BEFORE banding/grading*

5.1.1 CANNERS WITH CANNERS () **YES** () **NO**

5.1.2 CANNERS WITH MARKET () **YES** () **NO**

5.1.3 CANNERS WITH UNCERTAINS () **YES** () **NO**

5.1.4 MARKETS WITH MARKETS () **YES** () **NO**

5.1.5 MARKETS WITH UNCERTAINS () **YES** () **NO**

5. *Grading procedures on the boat (cont.)*

5.2 *Holding system BEFORE banding/grading*

5.2.1 CANNER size lobsters

() **Wooden crate** () **Plastic tote**

() **Barrel** () **Trays**

() **PVC tubes or similar** () **None**

() **Other:** _____

5.2.2 MARKET size lobsters

() **Wooden crate** () **Plastic tote**

() **Barrel** () **Trays**

() **PVC tubes or similar** () **None**

() **Other:** _____

5.2.3 UNCERTAIN size lobsters

() **Wooden crate** () **Plastic tote**

() **Barrel** () **Trays**

() **PVC tubes or similar** () **None**

() **Other:** _____

5.3 *Separate crates/totes/trays BEFORE grading/banding*

5.3.1 CANNER () YES () NO

5.3.2 MARKET () YES () NO

5.3.3 UNCERTAIN () YES () NO

5.4 *Partitioned crates/totes/trays BEFORE grading/banding*
 5.4.1 CANNER () YES () NO

5.4.2 MARKET () YES () NO

5.4.3 UNCERTAIN () YES () NO

5.5 *Overall handling of lobster*
 5.5.1 CANNER () Tossed () Placed

5.5.2 MARKET () Tossed () Placed

5.5.3 UNCERTAIN () Tossed () Placed

6. *Holding system AFTER banding/grading*

6.1 CANNER size lobsters
 () Wooden crate () Plastic tote
 () Barrel () Trays
 () PVC tubes or similar () None
 () Other: _____

6.2 MARKET size lobsters
 () Wooden crate () Plastic tote
 () Barrel () Trays
 () PVC tubes or similar () None
 () Other: _____

7. *Tarps*

7.1 *While fishing*
 () YES () NO
 () Other: _____

7.2 *After fishing (on the way back to the wharf)*
 () YES () NO
 () Other: _____

8. *Tank system*

() None () Plastic tub
 () Fibreglass tank () Other: _____

8.1 *Lid on the tank*

8.1.1 *While fishing* () None () 1/2 ON
 () 3/4 ON () Totaly ON

8.1.2 *On the way back* () None () 1/2 ON
 () 3/4 ON () Totaly ON

9. *Availability of water*

9.1 *While fishing*

9.1.1 CANNER None Flow-through Poured on Stagnant Other: _____9.1.2 MARKET None Flow-through Poured on Stagnant Other: _____9.2 *After fishing (on the way back to the wharf)*9.2.1 CANNER None Flow-through Poured on Stagnant Other: _____9.2.2 MARKET None Flow-through Poured on Stagnant Other: _____10. *Are the lobsters loose on the deck at some point?*10.1 CANNER YES NO10.2 MARKET YES NO11. *Maximum time the first lobster caught spent on the boat* (Check ONE) Less than two hours 2-4 hours 4-6 hours 6-8 hours 8-10 hours More than 10 hours12. *Minimum time the last lobster caught spent on the boat* (Check ONE) Less than two hours 2-4 hours 4-6 hours 6-8 hours 8-10 hours More than 10 hours13. *Packing over of the lobsters at the wharf?*13.1 CANNER NO DON'T KNOW YES By: Buyer Fisher Other: _____13.2 MARKET NO DON'T KNOW YES By: Buyer Fisher Other: _____14. *Dumping of the lobsters at the wharf?*14.1 CANNER NO DON'T KNOW YES By: Buyer Fisher Other: _____14.2 MARKET NO DON'T KNOW YES By:

15. How many traps were hauled on this day?

16. *Total landing*

16.1 CANNER _____ lbs

or _____ kg

16.2 MARKET _____ lbs

or _____ kg

17. Time the lobsters were landed at the wharf:

_____ hr _____ min. () am

() pm

18. **Other:** _____

Evaluation done by: _____

Date (Day/mth/yr): _____ / _____ / _____

Appendix N. Transportation conditions questionnaire.



LOBSTER HEALTH RESEARCH CENTRE
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Charlottetown, PE
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Tel.: (902) 566-0584
Fax: (902) 566-0851

File number: _____

Transportation Data

Date: (day/mo/yr) _____ / _____ / _____	
Facility/Plant Identification: Name: _____	
Location: _____	
Contact person: _____	
Tel.: (_____) _____	Fax: (_____) _____

1. *Description of the truck*

- Opened transportation compartment (pick-up truck)
- Closed transportation compartment
- Cap/tarp closed
- Refrigerated transportation compartment

2. *Outside temperature*

_____ °C, measured at (time of the day): _____ hour, _____ minutes

3. *Weather condition during transportation of the shipment*

<input type="checkbox"/> Sun	<input type="checkbox"/> Rain	<input type="checkbox"/> Night
<input type="checkbox"/> Clouds	<input type="checkbox"/> Snow	
<input type="checkbox"/> Mixture	<input type="checkbox"/> Mist	
<input type="checkbox"/> Other: _____		

4. *Speed of the wind*

5. *Shipment is in:*

() Crates
----- () Wood or () Plastic

() Plastic totes
----- () Lid or () No lid

() Other: _____

6. *Shipment on ice*

7. Transportation interval between wharf and processing plant

() *Less than 1 hr* () *1-2 hrs*
() *2-4 hrs* () *4-6 hrs*
() *6-8 hrs* () *Over 8hrs*
() *Do not know*

8. *Time the shipment spent in the transportation vehicle*

() *Less than 1 hr* () *1-2 hrs*
() *2-4 hrs* () *4-6 hrs*
() *6-8 hrs* () *Over 8hrs*
() *Do not know*

9. *Other observations influencing the health of this shipment*

Evaluation done by: _____

Date (day/mth/yr): / /

